STANDARD OPERATING PROCEDURE

Determination of Anions and Cations Extracted from PTFE® Filters by Ion Chromatography

Washington University in St. Louis
St. Louis, Missouri, USA

Prepared by: Crystal Weagle, Emily Stone and Emmie Le Roy
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1.0 SCOPE AND APPLICATION

The method described is used for the quantitative determination of anions (here defined as chloride (Cl^-), nitrite (NO_2^-), bromide (Br^-), nitrate (NO_3^-), sulfate (SO_4^{2-}), hydroxymethanesulfonate ($CH_3O_4S^-$ or HMS)) and cations (defined as sodium (Na^+), ammonium (NH_4^+), potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+})) in air samples collected on 25 mm PTFE filters. Each sampled filter is extracted with Milli-Q water and HPLC-grade methanol. The samples are then sonicated for 30 minutes. After sonication, 2 mL of each sample is transferred to a baked 4 mL glass vial and the remaining volume (3.5 – 4.0 mL) is transferred to a clean and dry 8 mL plastic amber vial. Extracts stored in glass vials are frozen immediately following extraction. Extracts stored in amber vials are refrigerated in sealed plastic bags and grouped by cartridge number. The refrigerated extracts in amber vials are analyzed for anions and cations using Ion Chromatography (IC).

REVISION HISTORY			
Revision No.	Change Description	Date	Authorization
1.0	Written for mesh Teflon and Nuclepore filters, includes filter cutting and 3 mL extractions.	December 12, 2018	Crystal Weagle
2.0	Edited for stretch PTFE® filters. Removal of filter cutting, addition of 6 mL extraction volume and preservation of filter after extractions.	January 25, 2019	Emily Stone Crystal Weagle
3.0	Updated for move to Washington University with new Integrion IC systems.	March 12, 2020	Emily Stone
4.0	Addition of HMS analysis, manual anion eluent preparation, HMS stock solution preparation, suppressor hydration.	November 26, 2020	Emmie Le Roy
4.1	Modified sonication method, addition of background subtraction	April 22 2022	Chris Oxford

2.0 SUMMARY OF METHOD

Once filters have returned from sampling in the field and have been analyzed through all other non-destructive methods (post-weighing, FTIR, HIPS, SSR, UV-Vis, XRF) they must then be extracted for IC and AMS analyses as the final step in the chemical analysis sequence. All filters are extracted using HPLC-grade methanol and 18 MΩ·cm ultrapure (Milli-Q) water, making it possible to analyze extracts for anions and cations using IC, and organics with an aerosol mass spectrometer (AMS). For analysis by IC, sample extracts pass through a column coated with quaternary ammonium active sites for anion analysis and through a column coated with carboxyl active sites for cation analysis. Ion separation occurs as extracts pass through the column due to different affinities of the various ions for the active sites. Following separation, the ions pass through a suppressor that lowers the background signal from ions in the eluent and increases the signal-to-noise ratio. Species are detected and quantified by a conductivity detector. Accuracy and precision of the method is monitored by routine analysis of quality control (QC) standards.

3.0 CONTAMINATION CONTROL

Contaminants in reagents, plastic and glass labware, pipette tips, and other components used in sample processing have the potential to cause erroneously high results. Therefore, all samples and standards are prepared using plastic and glass labware that has been thoroughly cleaned. As a portion of each extract is destined for organic analysis by AMS, all 25 mL and 4 mL borosilicate glass vials used during the extraction process are soaked in 1 % ACS-grade nitric acid for 24 hours, rinsed once with methanol, thrice with water, and then wrapped in aluminum foil and baked at 500 °C for 5 hours. Following extraction, sample storage vials will be capped and remain unopened unless for analysis. Extracts are then recapped immediately after the volume required for analysis is removed from the storage vial.

4.0 SAMPLE STORAGE AND RECORDKEEPING

4.1 Sample Storage

Filter samples are stored in petri dishes at room temperature prior to extraction and are extracted at room temperature. After extraction, the glass vials containing 2 mL of extract designated for AMS are frozen immediately at -20 °C. The remaining volume (~3.5 – 4.0 mL) from each filter extract is stored in a plastic, 8 mL amber vials and refrigerated at approximately 4 °C prior to analysis. Unused portions of sample extracts are stored for one year from the extraction date. After one year, one sample from each filter cartridge is stored long-term and the remaining samples are discarded.

4.2 E-Logs and Data Storage

Following extraction, the extraction volume, post-weigh date, and date of extraction are all recorded in the IC E-log.

5.0 EQUIPMENT, ELUENTS, AND STANDARDS

5.1 Laboratory Equipment

5.1.1 Labware

- Volumetric flasks: 10 mL, 25 mL, 250 mL, 1000 mL
- Pipette tips, plastic, disposable: 1- 100 µL, 10 – 1000 µL, 1 – 10 mL
- Dionex AS-DV Autosampler PolyVials (0.5mL) with Plain Caps
- Glass syringes: 100 µL, 5 mL
- 2 µm Polypropylene filter-heads
- Borosilicate glass vials: 25 mL (non-PTFE®-lined caps), 4 mL (PTFE®-lined caps)
- Plastic amber vials, 8 mL
- Borosilicate pipettes
- Pipette bulb
- PTFE®-coated tweezers
- Milli-Q squirt bottle
- Aluminum foil
- Spatula
- Weighing paper
- Glass working containers: HDPE, 500 mL
- Nalgene container: 500 mL, 50 mL

5.1.2 Equipment

- Dionex Integrion RFIC
- Micropipettes, variable volume
- Refrigerator (4 – 10 °C, nominal)
- Freezer (≤ - 18 °C, nominal)
- Ultrasonic bath
- 18 MΩ·cm ultrapure water (Millipore Sigma Milli-Q IQ 7000)
- Microbalance
- Thermo Furnace (with temperature reaching at least to 500°C)

5.1.3 Stock solutions and chemicals

- Dionex Six Cation-II Standard (Product No. 046070)
- Dionex Seven Anion Standard (Product No. 056933)
- Sodium Bicarbonate Concentrate
- Sodium Carbonate Concentrate
- Formaldehyde-sodium bisulfite adduct (solid sample)
  Or pre-prepared HMS stock solution (stable for 2 months at 4°C)
- Hydrochloric acid (high purity)

5.2 Cleaning and Preparation of Labware

5.2.1 Plastic amber vials

8 mL amber plastic vials are used to store IC extracts for up to 1 year.

- Rinse 8 mL amber vials once with ACS-grade methanol followed by a triplicate rinse with Milli-Q water.
- Place washed plastic labware on a Kimwipe® to air-dry overnight, and cover with a Kimwipe® to prevent dust settling on clean labware.
- Store clean and dry vials in sealed plastic bags labeled “Clean”.

5.2.2 Borosilicate vials: 25mL and 4mL and Glass working containers

- Place all borosilicate glassware and vials in a 1% ACS-grade nitric acid bath (use baskets for more convenient handlings) and allow to soak for 24 hours. Rinse all items with Milli-Q water, once with ACS-grade methanol followed by three additional rinses with Milli-Q water.
- Place glassware on a sheet of aluminum foil and cover all items with a Kimwipe® and allow them to dry overnight.
- All 25 mL vials and 4 mL vials are separated into sets of nine, each set of 9 vials wrapped in aluminum foil. Each pack of vials is bakes at 500°C for 5 hours.
- Once baked, glass vials remain in the aluminum foil pouches and are placed inside a sealed plastic bag with “Clean and Baked” and the date which they finished the baking procedure is written on the exterior of the bag with permanent marker.

5.2.3 Volumetric flasks & borosilicate pipets

- Rinse 25 mL volumetric flasks used for making standards six times with Milli-Q water. Store flasks upside down in a flask stand to allow water to drain.
- Rinse 250 mL and 1000 mL volumetric flasks for preparing eluent three times with Milli-Q water. Store flasks upside down in a flask stand to allow water to drain.
- Rinse borosilicate pipettes three times with Milli-Q water prior to use.

5.2.4 Glass Syringes

- Remove the plungers from the 5 mL glass syringes and rinse both pieces (the syringe and the plunger) once with ACS-grade methanol and then in triplicate with Milli-Q water in a shallow Tupperware® container. Place the plungers back in the syringes and push 3 full volumes of Milli-Q water through each syringe.
- For the 100 µL syringes used exclusively for HPLC-grade methanol, rinsed in triplicate with HPLC-grade methanol prior to each use.
- Leave all syringes to dry on a Kimwipe® or on a clean sheet of aluminum foil. Make sure to separate the plungers from the syringes to enable drying.
- When dry, wrap the syringes in a clean (or newly baked) sheet of aluminum foil. Then place the aluminum-wrapped syringes in a plastic bag labeled "Clean" with the date.

5.2.5 Polypropylene Filter-heads

Polypropylene filter heads are only used once before discarding. They are not cleaned before use.

5.2.6 Autosampler Vials and Pipette Tips

Autosampler vials for use with Thermo Scientific equipment are available commercially and are used without prior rinsing. Disposable pipette tips for use with micropipettes are available commercially and are also used without rinsing. If quality control blank analyses consistently show measurable ions, contamination due to autosampler vial and/or pipette tip may be considered.

5.3 Eluent and Eluent Cartridges

5.3.1 Manual preparation of anion eluent

For the anion chromatography system, eluent is manually prepared using sodium carbonate and sodium bicarbonate concentrated stock solutions. The eluent used is a 2.7 mM sodium carbonate (Na_2CO_3) / 0.3 mM sodium bicarbonate ($NaHCO_3$) solution.

- Thoroughly rinse the 1000 mL volumetric flask with Milli-Q water.
- Fill the flask half-way with Milli-Q (~500 mL).
- Add 5.4 mL of Na2CO3 and 0.6 mL of NaHCO3 to the flask.
- Gently swirl the flask, holding the neck above the fill line and using a Milli-Q squirt bottle fill the rest of the flask to the fill line.

5.3.2 Automatic generation of cation eluent

For the cation chromatography system, an eluent generator cartridge (Dionex EGC III MSA) is used to generate methanesulfonic acid eluent through electrolysis using only 18 MΩ·cm ultrapure water, therefore no manual preparation of eluent is needed.

5.4 Calibration Standards

A minimum of 16 calibration standards are prepared for anion IC analysis and 8 calibration standards are prepared for cation IC analysis and as outlined in section 5.4.3 and 5.4.4, respectively. These standards will either be used that day or refrigerated for use within the next seven days. If stored for longer than seven days, the prepared standards are discarded and standards are remade.

5.4.1 Anion/Cation Stock Solution

The anion and cation stock solutions are purchased directly from Dionex / Thermo Fisher (Dionex Seven Anion Standard (Product No. 056933) and Dionex® Six Cation-II Standard (Product No. 046070)).

5.4.2 Preparation of HMS Stock Solution

For anion analysis, a stock solution of 150 mg/L hydroxymethanesulfonate (HMS: $HOCH_2SO_3^-$) acidified to pH 3 with hydrochloric acid must be prepared from formaldehyde-sodium bisulfite adduct (purity 95%).

Note: **DO NOT** put spatula directly in chemical bottle!

- Pour a small amount (less than pea-sized) formaldehyde-sodium bisulfite adduct onto weighing paper by gently tapping the bottle on its side.
- Fold a separate piece of weighing paper diagonally and place on scale.
- Close draft shields and tare scale with weighing paper. Re-open draft shields.
- Wipe spatula with a Kimwipe® before using. Use spatula to transfer 37.5 mg (0.0375 g) of solid sample from the weighing paper to the folded weighing paper on the scale.
- Close draft shields. Record the actual weight of solid sample measured (i.e. 37.4-37.6 mg) in lab notebook. You should aim to be at least within 5% of 37.5 mg (35.6 – 39.4 mg).
- Transfer measured solid sample to a dry 250mL volumetric flask.
- Add ~100mL of Milli-Q water to 250 mL flask. Rinse the neck of the flask to wash down all solvent. Swirl gently by hand until solute is completely dissolved in solution. The heat of your hand can cause the glass to expand and volumes to be inaccurate, so only hold the flask only above the fill line.
- Under the fume hood, add 20 µL of high-purity HCl to the flask. Swirl gently by hand. Add Milli-Q water to the flask until you approach the fill line.
- Use a **clean** borosilicate pipet to add Milli-Q water drop-by-drop until the bottom of the meniscus reaches the fill line.
- Transfer contents of a 250 mL flask to a 500 mL Nalgene bottle. HMS is stable for up to 2 months at 4°C and is stored for two months if not used. When preparing standards, it is advisable to transfer 50 mL of HMS stock solution to a smaller bottle that you can take in and out of the fridge, leaving the remained of the HMS stock solution in the Nalgene bottle. Please write the date of preparation

of the stock solution on the bottle so it is clear to users when the solution will 'expire' and should be prepared anew.

5.4.3 Anion Calibration Standards

Anion calibration standards are prepared by diluting the Dionex Seven Anion Standard (Product No. 056933) and the stock solution of HMS in 18 MΩ·cm ultrapure water (Milli-Q) as shown in Tables 1 and 2. The resulting concentrations of the anions in each standard solution are shown in Table 3.

Table 1. Preparation method summary for inorganic anion calibration standards

Standard Label	Method of preparation
STD 3.0	750 µL anion stock solution in 25 mL flask
STD 2.0	500 µL anion stock solution in 25 mL flask
STD 1.5	375 µL anion stock solution in 25 mL flask
STD 1.0	250 µL anion stock solution in 25 mL flask
STD 0.75	188 µL anion stock solution in 25 mL flask
STD 0.5	125 µL anion stock solution in 25 mL flask
STD 0.25	63 µL anion stock solution in 25 mL flask
STD 0.1	2.00 mL of STD 0.5 in **10 mL** flask

Table 2. Preparation method summary for HMS calibration standards

Standard Label	Method of preparation
HMS 3.0	750 µL HMS stock solution in 25 mL flask
HMS 2.0	500 µL HMS stock solution in 25 mL flask
HMS 1.5	375 µL HMS stock solution in 25 mL flask
HMS 1.0	250 µL HMS stock solution in 25 mL flask
HMS 0.75	188 µL HMS stock solution in 25 mL flask
HMS 0.5	125 µL HMS stock solution in 25 mL flask
HMS 0.25	63 µL HMS stock solution in 25 mL flask
HMS 0.1	2.00 mL of STD 0.5 in **10 mL** flask

Table 3. Final anion concentrations in calibration standards (µg/mL)

Standard Label	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	PO_4^-	SO_4^{2-}	HMS
STD 3.0	0.60	0.90	3.00	3.00	3.00	4.50	4.50	4.50
STD 2.0	0.40	0.60	2.00	2.00	2.00	3.00	3.00	3.00
STD 1.5	0.30	0.45	1.50	1.50	1.50	2.25	2.25	2.25
STD 1.0	0.20	0.30	1.00	1.00	1.00	1.50	1.50	1.50
STD 0.75	0.15	0.225	0.75	0.75	0.75	1.125	1.125	1.125
STD 0.5	0.1	0.15	0.50	0.50	0.50	0.75	0.75	0.75
STD 0.25	0.05	0.075	0.25	0.25	0.25	0.375	0.375	0.375
STD 0.1	0.02	0.03	0.10	0.10	0.10	0.15	0.15	0.15

5.4.4 Cation Calibration Standards

Cation calibration standards are prepared directly from the Dionex Six Cation-II Standard (Product No. 046070) as described in Table 4. Milli-Q water is used to dilute to the volume specified in Table 3. The resulting concentrations of the cations in each standard solution are shown in Table 5.

Table 4. Preparation method summary for cation calibration standards

Standard Label	Method of preparation
STD 3.0	300 µL cation stock solution in 25 mL flask
STD 2.0	200 µL cation stock solution in 25 mL flask
STD 1.5	150 µL cation stock solution in 25 mL flask
STD 1.0	100 µL cation stock solution in 25 mL flask
STD 0.75	75 µL cation stock solution in 25 mL flask
STD 0.5	50 µL cation stock solution in 25 mL flask
STD 0.25	25 µL cation stock solution in 25 mL flask
STD 0.1	2.00 mL of STD 0.5 in **10 mL** flask

Table 5. Final cation concentrations in calibration standards (µg/mL)

Standard Label	Li^+	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
STD 3.0	0.60	2.40	3.00	6.00	3.00	6.00
STD 2.0	0.40	1.60	2.00	4.00	2.00	4.00
STD 1.5	0.30	1.20	1.50	3.00	1.50	3.00
STD 1.0	0.20	0.80	1.00	2.00	1.00	2.00
STD 0.75	0.15	0.60	0.75	1.50	0.75	1.50
STD 0.5	0.10	0.40	0.50	1.00	0.50	1.00
STD 0.25	0.05	0.20	0.25	0.50	0.25	0.50
STD 0.1	0.02	0.08	0.10	0.20	0.10	0.20

5.4.5 Quality Control Standards

An intermediate range anion and cation quality control (QC) standard is prepared using the Dionex Seven Anion Standard, HMS stock solution and Dionex Six Cation-II Standard stock solutions, as described in Table 6. Only Milli-Q water is used to dilute to the volume specified in Table 6.

Table 6. Preparation method summary for anion and cation calibration standards

Standard Label	Method of preparation
Anion QC STD 1.25	313 µL anion stock solution in 25 mL flask
HMS QC STD 1.25	313 uL HMS stock solution in 25 mL flask
Cation QC STD 1.25	125 µL cation stock solution in 25 mL flask

6.0 SAMPLE PREPARATION

6.1 Filter Extraction Procedure

- Label 25 mL glass vials and 4 mL glass vials with the filter label of the cartridge(s) to be extracted using labeling tape. Label the 8 mL plastic amber vials using silver permanent marker with the filter label of the cartridge(s) to be extracted.
- For each cartridge, one lab blank will also be prepared and labeled according to the cartridge number (e.g. ILNZ-027-LB). The lab blanks are prepared following the same procedure as filter extracts.
- Using PTFE®-coated tweezers, place each filter the corresponding labeled 25 mL glass vial.
- Using a 100 µL glass syringe, transfer 240 µL of HPLC-grade methanol directly onto the filter in each glass vial.
- Using a 5 mL glass syringe, transfer 5.8 mL of Milli-Q water into each 25 mL glass vial.
- Using the PTFE®-coated tweezers, place a baked aluminum foil square over the top of the 25 mL glass vial and tightly screw on the cap. The foil square prevents any liquid from touching the inside of the unbaked, plastic caps which may contaminate the sample and interfere with organic analyses.
- Place the 25 mL glass vials in sonication bath tray. Before sonication, check for optimum water level. Sonicate for 30 minutes. Remove the vials from the ultrasonic bath.
- Using a new 5 mL glass syringe and 2 µm Polypropylene filter-head for each sample, transfer 2 mL of each extract to the baked 4 mL glass vials. Make sure that the label on the 4 mL vial matches the label on the 25 mL vial. Be sure to put the 2 µm Polypropylene filter-head on the tip of the syringe AFTER the 2 mL aliquot is inside the syringe. Filter SLOWLY and ensure that the filter head is not punctured while you extract the sample. Pushing the syringe needles too quickly will result in improper filtration and could puncture the filter.
- Using the 5 mL glass syringe and 2 µm Polypropylene filter-head, transfer the remaining volume in the 25 mL glass vial (3.5 – 4.0 mL) to the 8 mL plastic amber vial. Make sure that the label on the 8 mL amber vial matches the label on the 25 mL vial.
- Filter remnants are discarded after extraction.

6.2 Sample Storage

- The 2 mL aliquot of the extract that is stored in a 4 mL glass vial is to be frozen immediately following extraction. Wrap the cap with approximately 10 cm of PTFE® tape, then lay horizontally for freezing to prevent the vial from bursting.
- The remaining extract in the 8 mL plastic amber vials will remain refrigerated until analysis and for a minimum of 1 year.

7.0 ANALYSIS BY ION CHROMATOGRAPHY

7.1 System Shut Down and Start-Up

If the system will not be used for longer than 4 days, it will be necessary to remove the columns, run water through the system and plug the suppressors. The water rinse is particularly important for the carbonate/bicarbonate system to prevent crystallization. For the MSA system, the water rinse will help prevent acid degradation.

If the system has not used for longer than 4 days, and has been properly shut down, it will be necessary to re-connect the guard column, analytical column and suppressor. If starting analysis after a long period of inactivity (more than 1 month), the suppressors should be hydrated. Follow the directions in the instrument manual for connecting columns and suppressor and for instructions on hot to hydrate the suppressor.

7.2 SPARTAN Instrument Method

- Typically, 50 samples (including waters, blanks, standards, and filter extracts) complete an IC batch.
- The analysis will be set up to run a complete calibration curve at the beginning of each anion and cation IC batch. One 18 MΩ·cm deionized water blanks should be run prior to the calibration curve for sample loop rinsing.
- The QC standard will be run following the calibration standards, at the end of the sample queue, and after every 10-12 samples to ensure instrument stability.
- Waters ((18 MΩ·cm) deionized water) will be run intermittently throughout each IC batch for sample loop rinsing and assessment of contamination.
- Baseline subtraction must be assigned with every calibration and cartridge. For the calibration, the baseline should be a water sample halfway into calibration. For each cartridge, the baseline should be the lab blank.
- The Dionex Chromeleon software is set up to use a linear function to produce a calibration curve for all anions. The Dionex Chromeleon software is set up to use a linear function to produce a calibration curve for all cations, except ammonium, which uses a

cubic function. Peak areas obtained for each ion in each sample are converted to concentration (µg/mL) using the calibration curve obtained for each IC batch.

8.0 DATA VALIDATION

8.1 Level 1 Data Validation

Level 1 data validation of each IC batch involves manual inspection of each chromatogram in the IC batch. After each IC batch is complete, each chromatogram is examined for:

- Proper peak identification by the Dionex Chromeleon software.
- If necessary, the peak shapes are corrected, and integration windows adjusted.
- Peak overlaps are examined and corrected when possible.
- Proper baseline subtraction for each calibration and cartridge

Additional level 1 data validation checks for each IC batch include:

- All samples fall within the range of the standards used for the IC batch.
- Investigation and flagging of outliers (e.g. large sulfate peak).
- Examining the consistency between QC standards and the calibration standards.
- The correlation coefficient is > 0.995 for all relevant ions.
- The peak areas in water blanks do not exceed 10*MDL for relevant ions.

8.2 Level 2 Data Validation

After both anion and cation data are collected in the master data base for a given filter, level 2 data validation is performed based on known physical relationships:

- Comparison of the new values for consistency with long-term concentrations at a given sampling site and other close data points in the time series.
- Linearity between ammonium and the sum of sulfate and nitrate for filters in each cartridge.
- When possible, comparison with concentrations of the same species measured by a different method (e.g. XRF).
- Comparison of the mass concentration for a filter to the sum of measured chemical species.