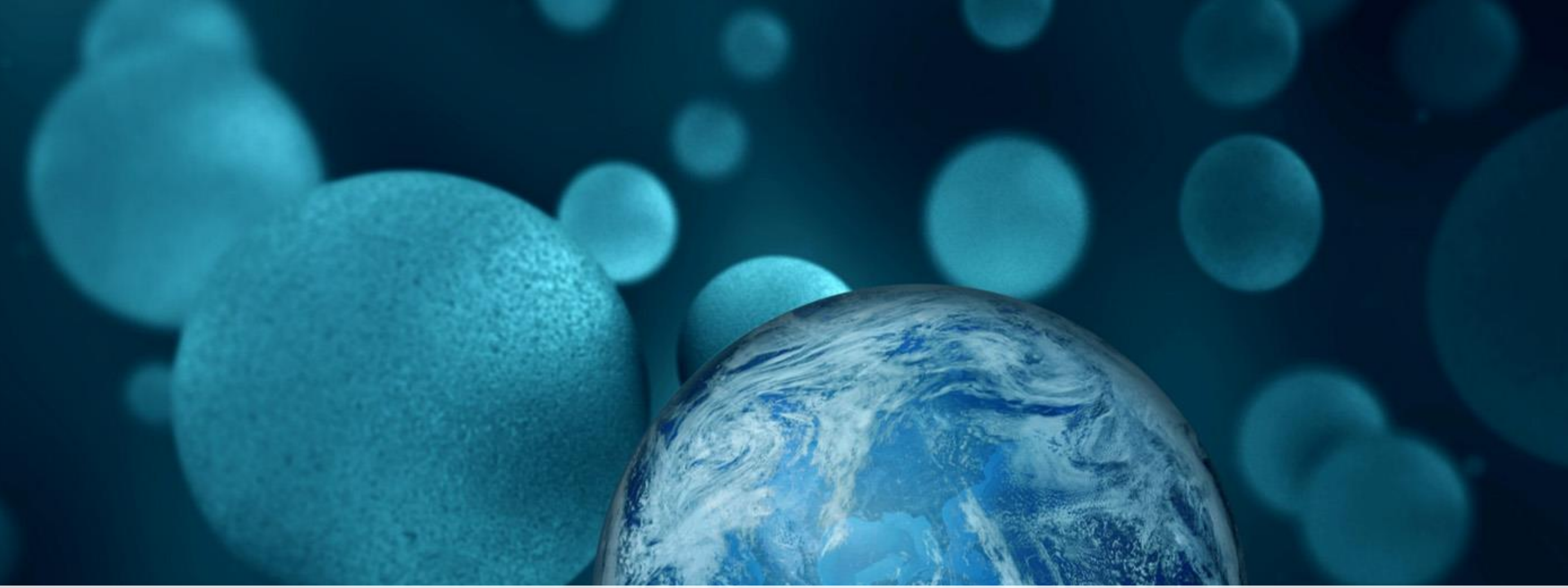

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ASTM D8001: A New Method for “Total Nitrogen” and Total Phosphate using Ion Chromatography

Richard F. Jack, Ph.D.
Thermo Scientific

EPA interest in Total N and Total P

We have a Problem!

ORSANCO

- A coalition in Ohio Valley focusing on total nutrient monitoring

- **The Program:**
- bi-weekly monitoring for algal counts and genus-level identification biweekly monitoring of total phosphorus, ammonia-nitrogen, TKN, and nitrite + nitrate-nitrogen biweekly monitoring of chlorophyll-a
- **The Benefits:**
- provides consistent enumeration and identification of algae establishes river-wide trends in algae composition and abundance provides early warning capabilities to downstream utilities for algae conditions provides data for the future development of nutrient criteria
- Currently, seven water utilities participate in the algae & nutrient monitoring program. Each participant assumes responsibility for collecting raw water samples on a bi-monthly basis and submitting them to the laboratories.
- EA2 Water Systems, Evansville, IN
Louisville Water Company, Louisville, KY
Northern Kentucky Water District, Ft. Thomas, KY
Paducah Water Works, Paducah, KY
Participating Water Utilities:
West View Water Authority, Pittsburgh, PA
Wheeling Water Treatment Plant, Wheeling, WV
WV-American Water Company, Huntington, WV

Definitions relevant to the determination of N

- Total Nitrogen – inorganic nitrogen plus organic nitrogen
- Total Nitrogen – particulate nitrogen plus dissolved nitrogen
- Particulate Nitrogen - A significant amount of nitrogen (~20%) is bound to insoluble organic matter. This nitrogen can eventually oxidize to inorganic nitrogen becoming bioavailable.
- Dissolved Nitrogen – dissolved organic nitrogen plus dissolved inorganic nitrogen
- Dissolved Organic Nitrogen – soluble organic compounds containing nitrogen, examples are humic acids, urea, glycine, nicotinic acid, etc.
- Total Inorganic Nitrogen – ammonium ion, nitrate ion, and nitrite ion. These ions are freely water soluble.
- Dissolved Inorganic Nitrogen = Total Inorganic Nitrogen

EPA Methods for Determining Total Nitrogen and Phosphorus

- Nitrogen Methods
  - EPA Methods 351.2, 351.4, 353.2
    - EPA 351.2/351.4—TKN
    - EPA 353.2—Nitrate/nitrite (colorimetric, cadmium reduction)
- Total Phosphorus
  - EPA Methods 365.2 and 365.4
    - EPA 365.2—Phosphorus, all forms (colorimetric)
    - EPA 365.4—Total phosphorus (colorimetric)

Definition issues Measuring Nitrogen and Total Nitrogen

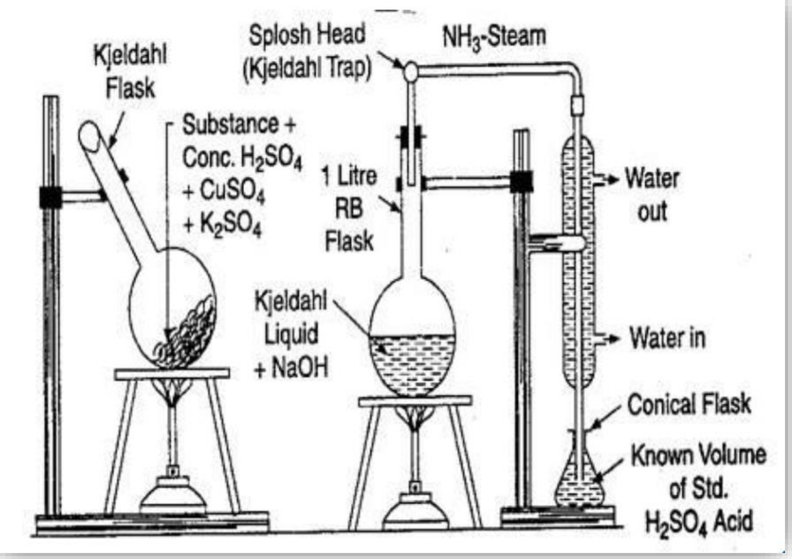
- The currently accepted (EPA approved) “method” for Total Nitrogen (TN) is the sum of Total Kjeldahl Nitrogen (TKN) and Nitrate plus Nitrite Nitrogen (NOx).
  - There is no EPA approved method for the determination of TN as a single technique
- The determination of TN is as TKN + NOx is problematic:
  - When NOx exceeds the TKN concentration the TKN result can be low (negative bias).
  - In some matrices NOx can be partially measured as TKN (positive bias).
- States are requiring TN in permits without clearly defining what TN is.

Total Kjeldahl Nitrogen

- **Digestion – Distillation – Capture - Titration**
- Total Kjeldahl Nitrogen (TKN) - Determines organic nitrogen and ammonium ion. The TKN method does not include Nitrogen from Nitrate or Nitrite.
- TKN methods digest samples with an excess of concentrated sulfuric acid. A metallic catalyst, previously mercury but lately copper, is added to speed the digestion and improve recovery. Potassium sulfate is added to raise the boiling point to 380 ºC. The digestion converts organic nitrogen to ammonium ion.

- **Advantages of the TKN digestion**
- Complete recovery in samples containing particulate matter and high organic loads
- Years of historical data
- Numerous methods

- **Disadvantages of TKN**
- Dangerous (boiling /fuming sulfuric acid solutions)
- Only determines organic nitrogen and ammonium ion
- Biased results for samples containing nitrate
- May partially recover some nitrate



Nitrogen Methods and Total P

Common Name		Method description	Method*
Total Ammonia, Ammonia-Nitrogen	Direct Measurement	Colorimetric, automated phenate	EPA 350.1
Nitrite	Direct Measurement	Ion Chromatography	EPA 300.1(A)
Nitrate	Direct Measurement	Ion Chromatography	EPA 300.1(A)
	Direct Measurement	Spectrophotometric Measurement of Nitrate in Water and Wastewater	Hach 10206
Nitrate+Nitrite	Direct Measurement	Colorimetric, cadmium reduction, automated	EPA 353.2
Organic Nitrogen	Calculated	Total Kjedahl Nitrogen (TKN) minus Total Ammonia	EPA 351.2 minus EPA 350.1
Total Inorganic Nitrogen	Calculated	Nitrate + Nitrite plus Total Ammonia	EPA 353.2 plus EPA 350.1
Total Kjeldahl Nitrogen	Direct Measurement	Colorimetric, semi automate, block digester	EPA 351.2, SM 4500, ASTM
	Direct Measurement	Simplified Spectrophotometric Measurement of Total Kjeldahl Nitrogen in Water and Wastewater	Hach 10242
Total Nitrogen	Calculated	Total Kjedahl Nitrogen plus Nitrate+Nitrite	EPA 351.2 plus EPA 353.2
	Direct Measurement	Alkaline persulfate digestion plus NOx	SM 4500-N-C
	Direct Measurement	Lachat persulfate digestion	Lachat 10-107-04-4
Total Phosphorus	Direct Measurement	Colorimetric; acid persulfate digestion	EPA 365.1

* Not a complete list

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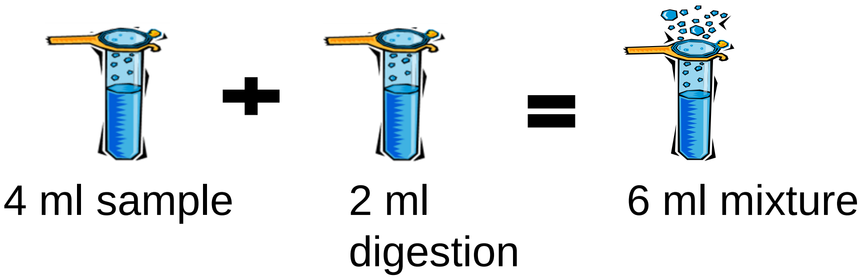
New Method for Total N, Total P and TKN

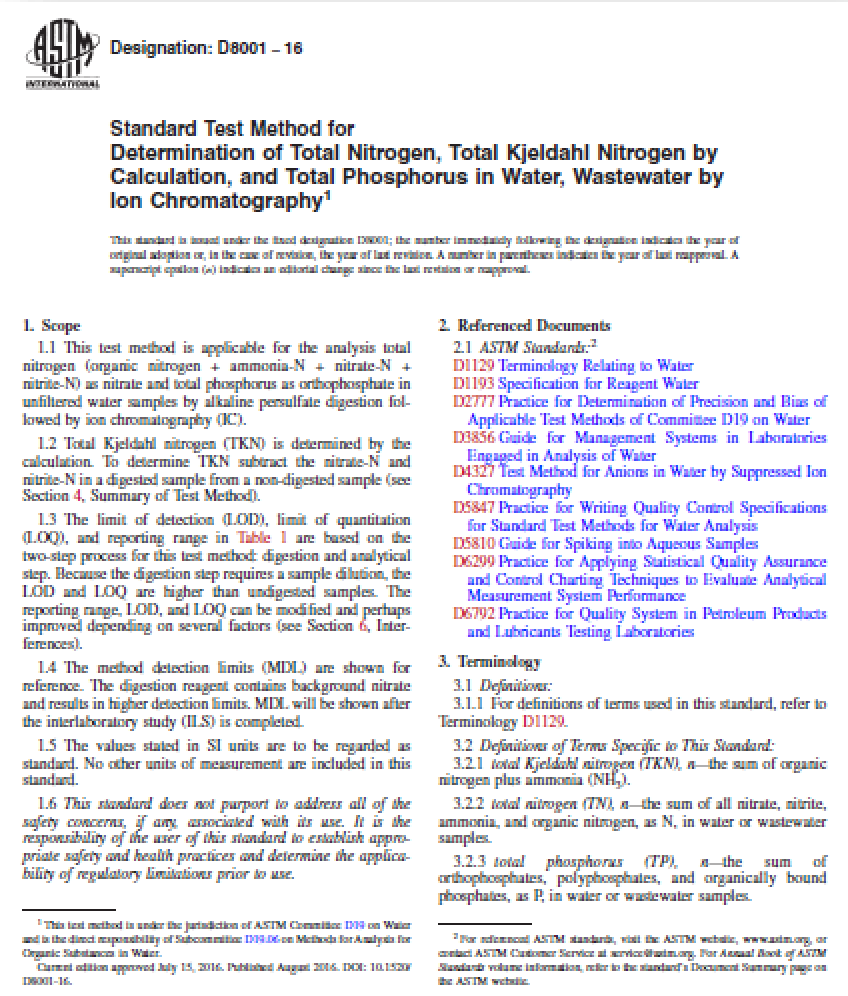
- Digestion
  - REALLY is a “Total N and P” Method
  - Easy, safe
- Analysis
  - Allows simultaneous analysis of N and P
  - Digested minus Undigested will give TKN

Method Aspects and Challenges

- Sample Preservation
- Digestion
  - Dilute to Digest = affects MDL
  - Interferences levels TOC, TDS
  - Particles vs. filtered samples?
  - How long to Digest (heating)
- Sample Analysis
  - Column capacity can be exceeded, affecting robustness, MDL
  - MDL
  - Robustness
  - Injection size
  - Runtimes



New ASTM D8001



Designation: D8001 – 16

Standard Test Method for Determination of Total Nitrogen, Total Kjeldahl Nitrogen by Calculation, and Total Phosphorus in Water, Wastewater by Ion Chromatography[1]

This standard is issued under the fixed designation D8001; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method is applicable for the analysis total nitrogen (organic nitrogen + ammonia-N + nitrate-N + nitrite-N) as nitrate and total phosphorus as orthophosphate in unfiltered water samples by alkaline persulfate digestion followed by ion chromatography (IC).

1.2 Total Kjeldahl nitrogen (TKN) is determined by the calculation. To determine TKN subtract the nitrate-N and nitrite-N in a digested sample from a non-digested sample (see Section 4, Summary of Test Method).

1.3 The limit of detection (LOD), limit of quantitation (LOQ), and reporting range in Table 1 are based on the two-step process for this test method: digestion and analytical step. Because the digestion step requires a sample dilution, the LOD and LOQ are higher than undigested samples. The reporting range, LOD, and LOQ can be modified and perhaps improved depending on several factors (see Section 6, Interferences).

1.4 The method detection limits (MDL) are shown for reference. The digestion reagent contains background nitrate and results in higher detection limits. MDL will be shown after the interlaboratory study (ILS) is completed.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water
D4327 Test Method for Anions in Water by Suppressed Ion Chromatography
D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis
D5810 Guide for Spiking into Aqueous Samples
D6299 Practice for Applying Statistical Quality Assurance and Control Charting Techniques to Evaluate Analytical Measurement System Performance
D6792 Practice for Quality System in Petroleum Products and Lubricants Testing Laboratories

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *total Kjeldahl nitrogen (TKN), n*—the sum of organic nitrogen plus ammonia (NH_3).

3.2.2 *total nitrogen (TN), n*—the sum of all nitrate, nitrite, ammonia, and organic nitrogen, as N, in water or wastewater samples.

3.2.3 *total phosphorus (TP), n*—the sum of orthophosphates, polyphosphates, and organically bound phosphates, as P, in water or wastewater samples.

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.
Current edition approved July 15, 2016. Published August 2016. DOI: 10.1520/D8001-16.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

- Formally approved by ASTM , not EPA.
- Digestion followed by IC
- Single lab validated
- 5 years for a multi-lab study
- Multiple matrices
- QA/QC Criteria
  - LOD, LOQ
  - Calibration range
  - Spike recoveries
- Robustness in hi TDS
- POTW and River waters
- Comparison w/ SM4500-N-C

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Scope and Method Summary

- This test method is applicable for the analysis total nitrogen (organic nitrogen + ammonia-N + nitrate-N + nitrite-N) as nitrate and total phosphorus as orthophosphate in unfiltered water samples by alkaline persulfate digestion followed by ion chromatography (IC).
- A water sample is digested with alkaline persulfate and heated.
  - Initial alkaline conditions oxidize dissolved/ suspended nitrogen to nitrate.
  - Over time the solution becomes acidic (pH ~2) resulting in the hydrolysis of dissolved / suspended phosphorus to orthophosphate.

$$S_2O_8^{2-} + H_2O \rightarrow 2HSO_4^- + \frac{1}{2} O_2$$

- The determinative step using IC is equivalent to Test Method D4327 / EPA 300.1.

5.0 Significance and Use

- 5.1 This test method allows the simultaneous determination of total nitrogen and total phosphorous from one sample digestion step.
- 5.2 This test method measures oxidized ammonia and organic nitrogen (as nitrate) and soluble nitrate simultaneously. By subtracting the nitrate + nitrite value from a non-digested sample gives a TKN:

$$TN = TKN + [(NO_3 - N) + (NO_2 - N)]$$

$$TKN = (NH_3 - N) + (Organic - N)$$

- Using D8001

$$icTKN = Digested - Undigested\ Sample$$

$$icTKN = TN - [(NO_3 - N) + (NO_2 - N)]$$

$$icTKN = D8001 - D4327\ or\ EPA\ 300.1$$

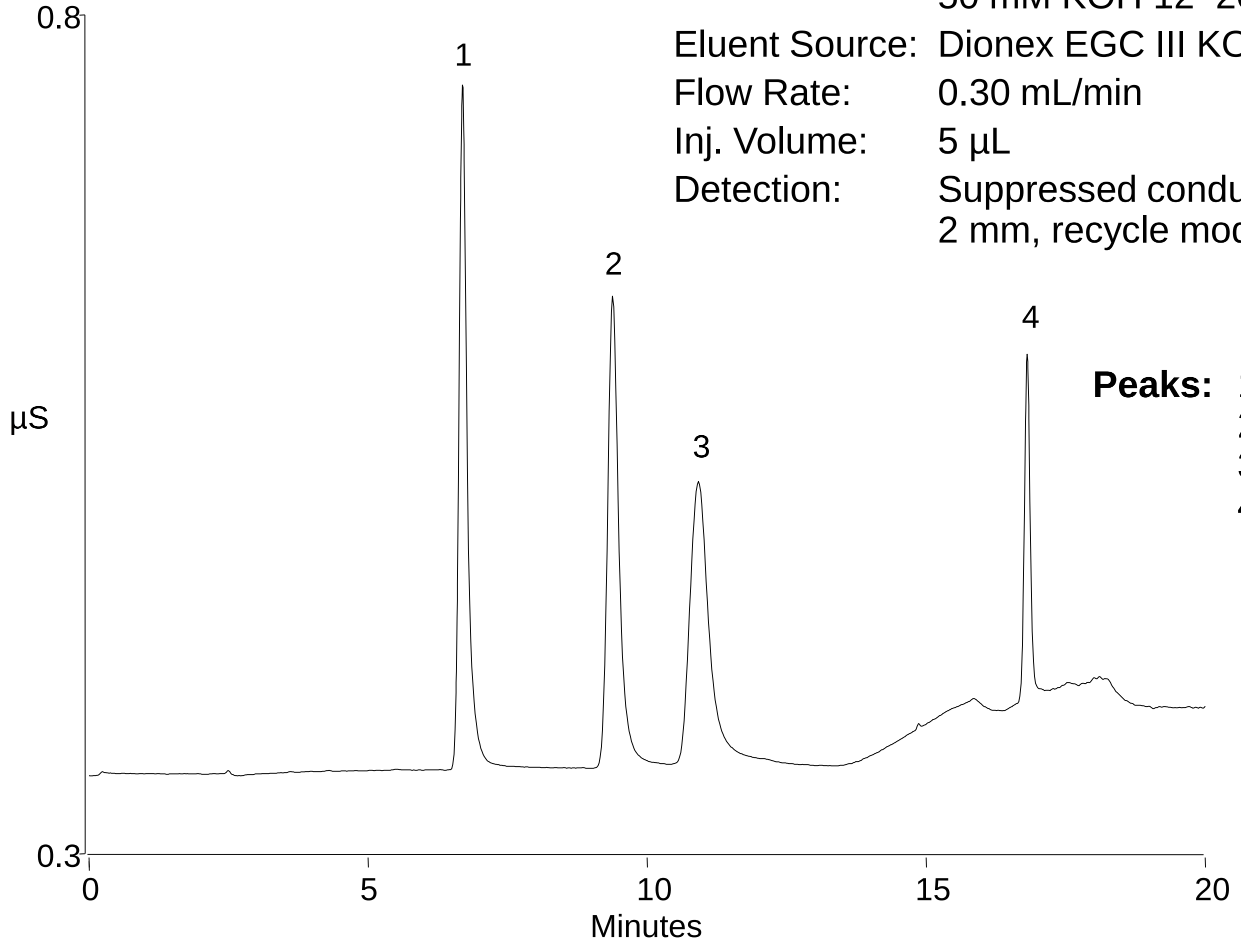
System

- Thermo Scientific Dionex ICS-2100 integrated RFIC system including:
  - Pump
  - Degasser
  - Eluent Generator
  - Column Heater
  - Dionex AS-AP Autosampler with 250 μL syringe
- Consumables
  - Thermo Scientific™ Dionex™ IonPac™ AG19/AS19 Anion-Exchange Column set
  - Thermo Scientific Dionex EGC III KOH Eluent Generator Cartridge
  - Thermo Scientific Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column
  - Thermo Scientific™ Dionex™ AERS™ Anion Electrolytically Regenerated Suppressor
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software for system control and data processing

Chromatographic Conditions

- **Columns:** Dionex IonPac AS19 Analytical, 2 x 250 mm
  Dionex IonPac AG19 Guard, 2 x 50 mm
- **Eluent Source:** Dionex EGC III KOH Cartridge with Dionex CR-ATC
- **Eluent:** 20 mM KOH 0–10 min, 20–50 mM 10–12 min,
  50 mM 12–20 min
- **Flow Rate:** 0.30 mL/min
- **Injection Volume:** 5 µL
- **Temperature:** 30 ^{o}C
- **Detection:** Suppressed conductivity, Dionex AERS suppressor,
  2 mm, recycle mode
- **Run Time:** 20 min

Standard Separation of Nitrite-N, Nitrate-N, and Phosphate-P

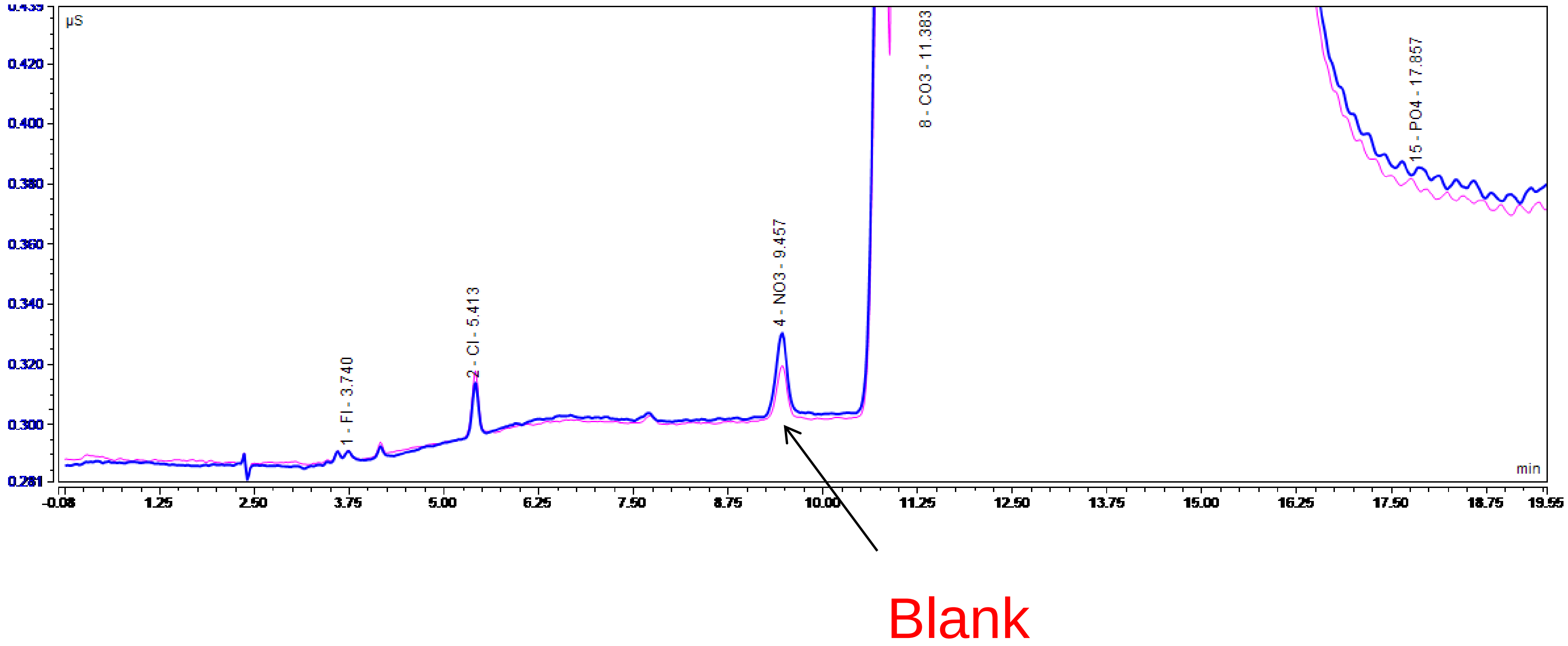


Column:	Dionex IonPac AG19/AS19, 2 mm
Eluent:	20 mM KOH 0–10 min, 20–50 mM KOH 10–12 min, 50 mM KOH 12–20 min
Eluent Source:	Dionex EGC III KOH with Dionex CR-ATC
Flow Rate:	0.30 mL/min
Inj. Volume:	5 µL
Detection:	Suppressed conductivity, Dionex AERS 500, 2 mm, recycle mode

Peaks:		
	1. Nitrite-N	100 µg/L
	2. Nitrate-N	100
	3. Carbonate	—
	4. Phosphate-P	100

Alkaline Persulfate Blank Injections

- MDL chromatograms – Reaction injection
- Blue 3.3 ppb NO_3-N spike
- Pink Reagent only



QA QC

Analyte	Calibration Range (µg/L)	Linearity[1] (r^2)	System LOD[2] (µg/L)	System LOQ[3] (µg/L)	Sample LOD (µg/L)	Sample LOQ (µg/L)
Nitrite-N	2.5–300	0.9999	0.76	2.5	—	—
Nitrate-N	2.5–300	0.9999	1.0	3.4	171	171
Phosphate-P	2.5–300	0.9998	1.3	4.2	19.5[4]	63[4]

[1] Ten calibration levels, each injected in duplicate

[2] Limit of detection (LOD) calculated as 3 × S/N

[3] Limit of quantificaiton (LOQ) calculated as 10 × S/N

[4] Phosphate LOD/LOQ was calculated based on a dilution factor of 15 × relative to the system concentrations

Digestion reagent has background NO_3

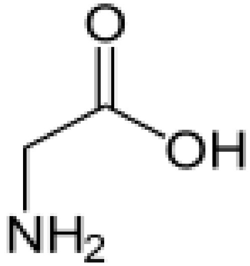
QA/QC – system MDL

	Nitrate		Nitrite		Phosphate
Injection	**Conc. injected 1.5 ug/L**	**Conc. injected 0.75 ug/L**	**Conc. injected 1.1 ug/L**	**Conc. injected 0.55 ug/L**	**Conc. injected 1.8 ug/L**
1	1.76	1.10	1.29	0.88	1.84
2	1.72	1.21	1.21	0.84	1.58
3	1.61	1.30	1.29	0.88	1.58
4	1.90	1.20	1.32	0.74	1.83
5	1.71	1.25	1.24	0.90	1.38
6	1.70	1.36	1.35	0.89	1.53
7	1.70	1.28	1.28	0.88	1.43
Avg.	1.7275	1.24125	1.2825	0.85875	1.59625
Std.	0.088021	0.083409	0.046803	0.055506	0.179523
MDL Undigested	0.297	0.260	0.146	0.146	0.563

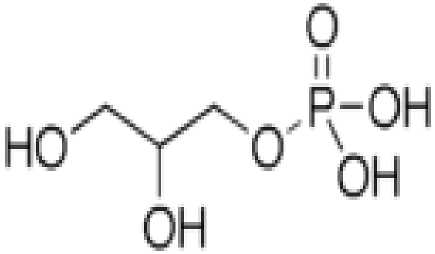
Evaluation of the N and P Recovery from Quality Control Standards

- Nitrogen-Containing Compounds
  - Nicotinic acid
  - Urea
  - Ammonium chloride
  - Glycine

- Phosphorus-Containing Compounds
  - Glucose-1-phosphate
  - Adenosine triphosphate
  - Phytic acid
  - Glycerophosphate



Glycine



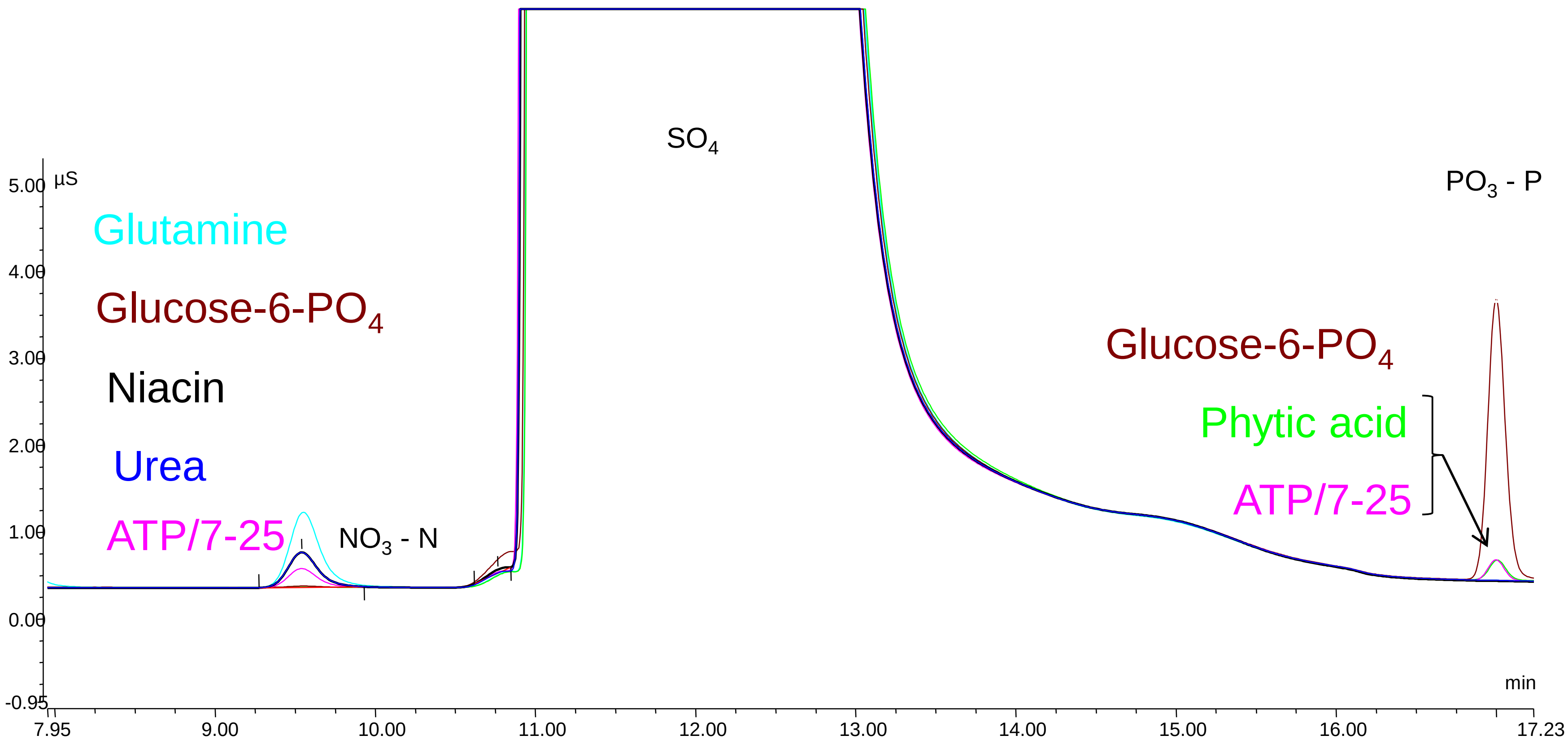
Glycerophosphate

Recovery of N and P from Quality Control Standards (Diluted 15×)

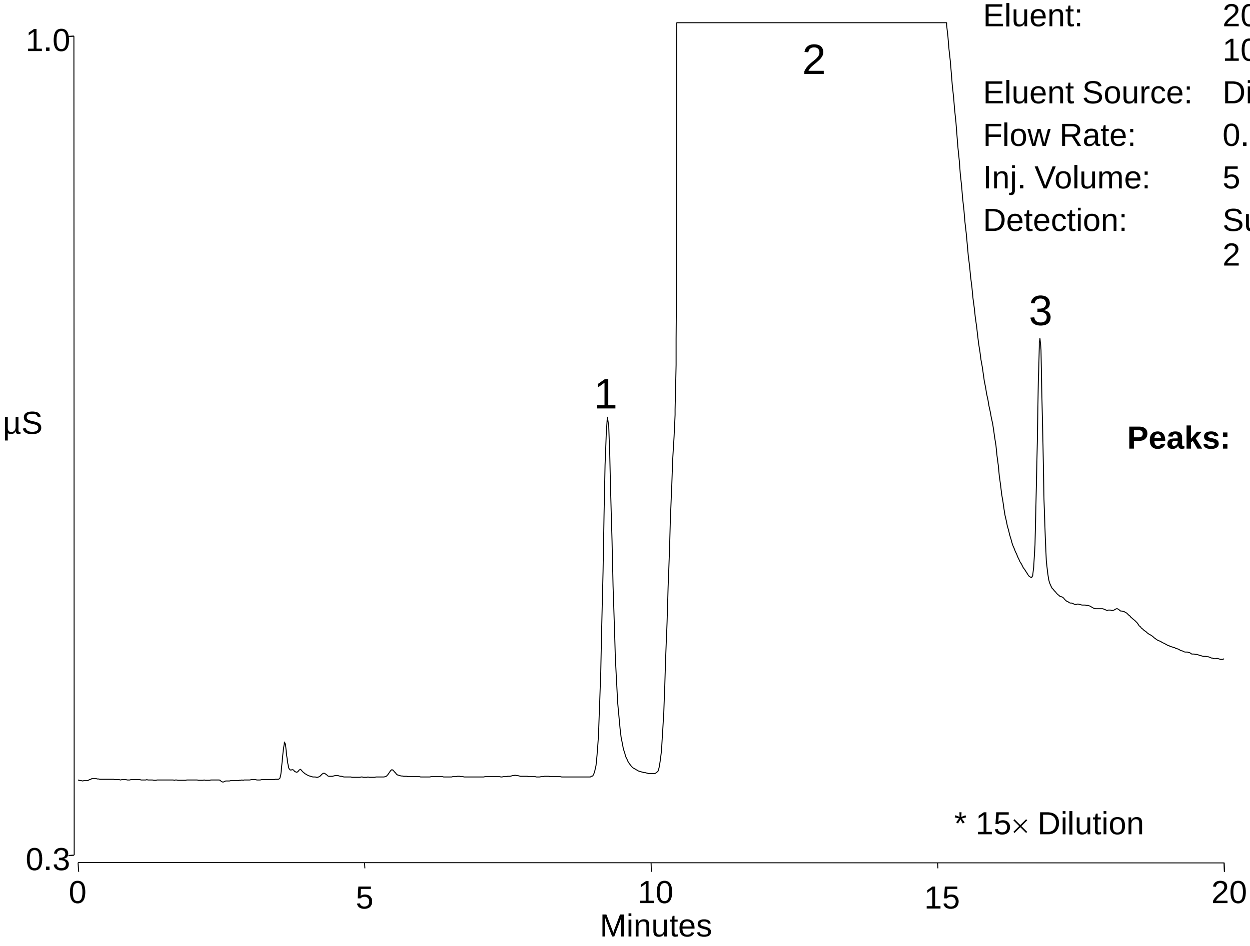
Nitrogen Compounds	Nominal Concn (mg N/L)	Expected Concn (mg N/L)	Found Concn (mg N/L)	% Recovery
Nicotinic Acid	1.95	0.129	0.129	100.1
Urea	2.02	0.133	0.127	95.4
Glycine	**1.49**	**0.098**	**0.094**	**95.6**
Ammonium Chloride	2.07	0.137	0.127	93.1
Phosphorus Compounds	**Nominal Concn (mg P/L)**	**Expected Concn (mg P/L)**	**Found Concn (mg P/L)**	**% Recovery**
Glucose-1-Phosphate	1.93	0.1290	0.1253	97.1
Adenosine Triphosphate	1.76	0.1162	0.099	85.2
Phytic Acid	1.86	0.1232	0.1052	85.4
Glycerophosphate	**1.63**	**0.1077**	**0.107**	**99.3**

Chromatogram of Digestion Test compounds

- spike recoveries



Glycine-N and Glycerophosphate-P after Alkaline Persulfate Digestion



Column:	Dionex IonPac AG19/AS19, 2 mm
Eluent:	20 mM KOH 0–10 min, 20–50 mM KOH 10–12 min, 50 mM KOH 12–20 min
Eluent Source:	Dionex EGC III KOH with Dionex CR-ATC
Flow Rate:	0.30 mL/min
Inj. Volume:	5 µL
Detection:	Suppressed conductivity, Dionex AERS 500, 2 mm, recycle mode

Peaks:		
	1. Nitrate-N	101 µg/L
	2. Sulfate	—
	3. Phosphate-P	105

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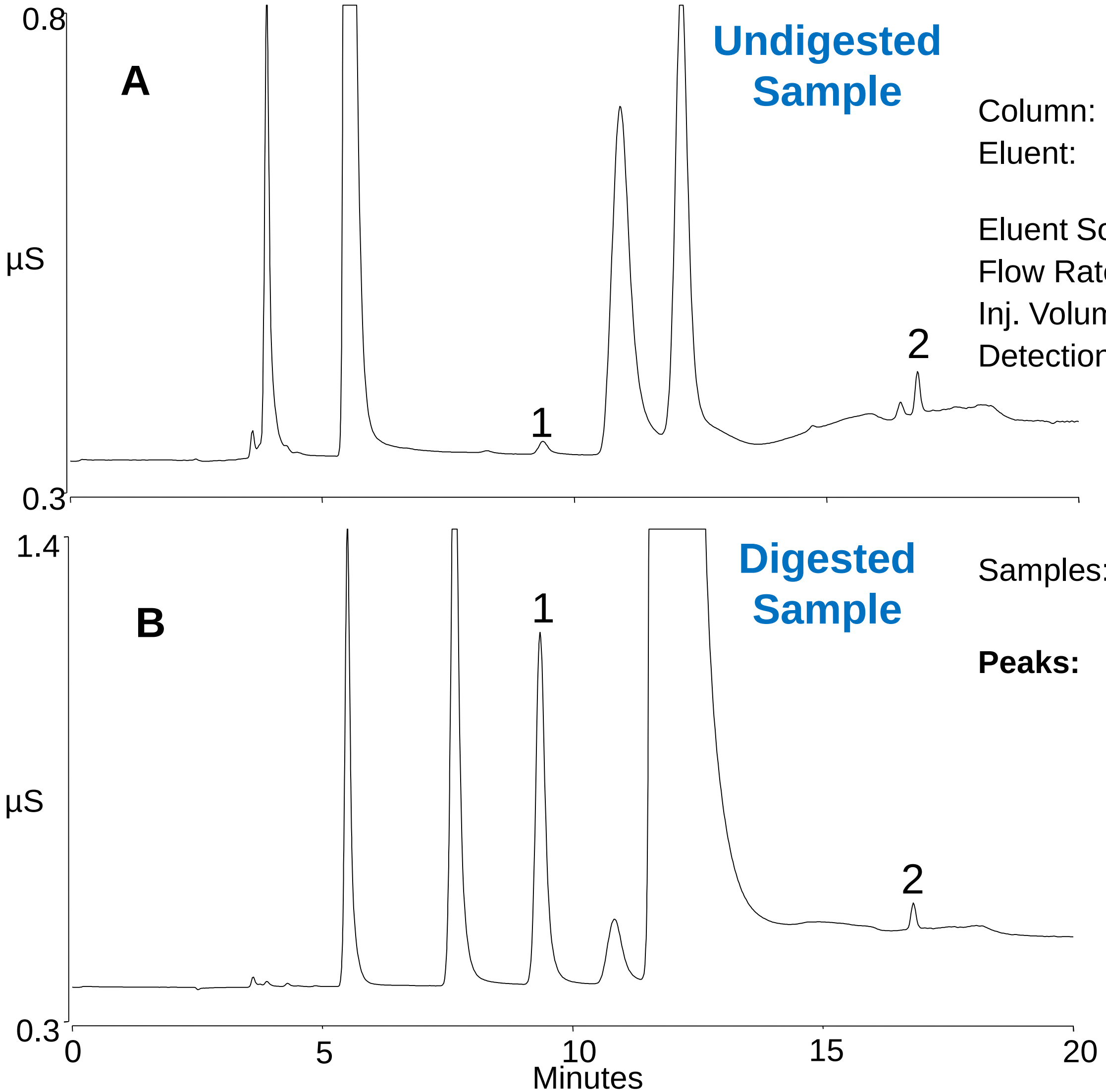
Comparison Between Undigested and Digested Raw Sewage



Column:	Dionex IonPac AG19/AS19, 2 mm
Eluent:	20 mM KOH 0–10 min, 20–50 mM KOH 10–12 min, 50 mM KOH 12–20 min
Eluent Source:	Dionex EGC III KOH with Dionex CR-ATC
Flow Rate:	0.30 mL/min
Inj. Volume:	5 µL
Detection:	Suppressed conductivity, Dionex AERS 500, 2 mm, recycle mode

Samples: A. Undigested raw sewage, 100× dilution
B. Digested raw sewage, 150× dilution

Peaks:	**A**	**B**
1. Nitrate-N	5.5	262 µg/L
2. Phosphate-P	22	30

Undigested and Digested Local Wastewater Sample Results

Sample[1]	Undigested (mg N/L)	Digested (mg N/L)	Undigested (mg P/L)	Digested (mg P/L)
SVL Effluent T3	6.52	8.54	2.85	3.20
SVL Primary Effluent	0.31	36.52	2.02	3.87
SJC Filtered Effluent	11.02	13.01	0.23	0.43
SJC Final Effluent	14.77	12.66	0.33	0.40
SJC TPS	11.69	13.43	0.25	0.39
SJC Raw Sewage	0.55	39.87	2.19	4.50

All results are calculated after accounting for the dilution factor

[1]SVL = Sunnyvale, CA; SJC = San Jose, CA

icTKN from various Wastewater Samples

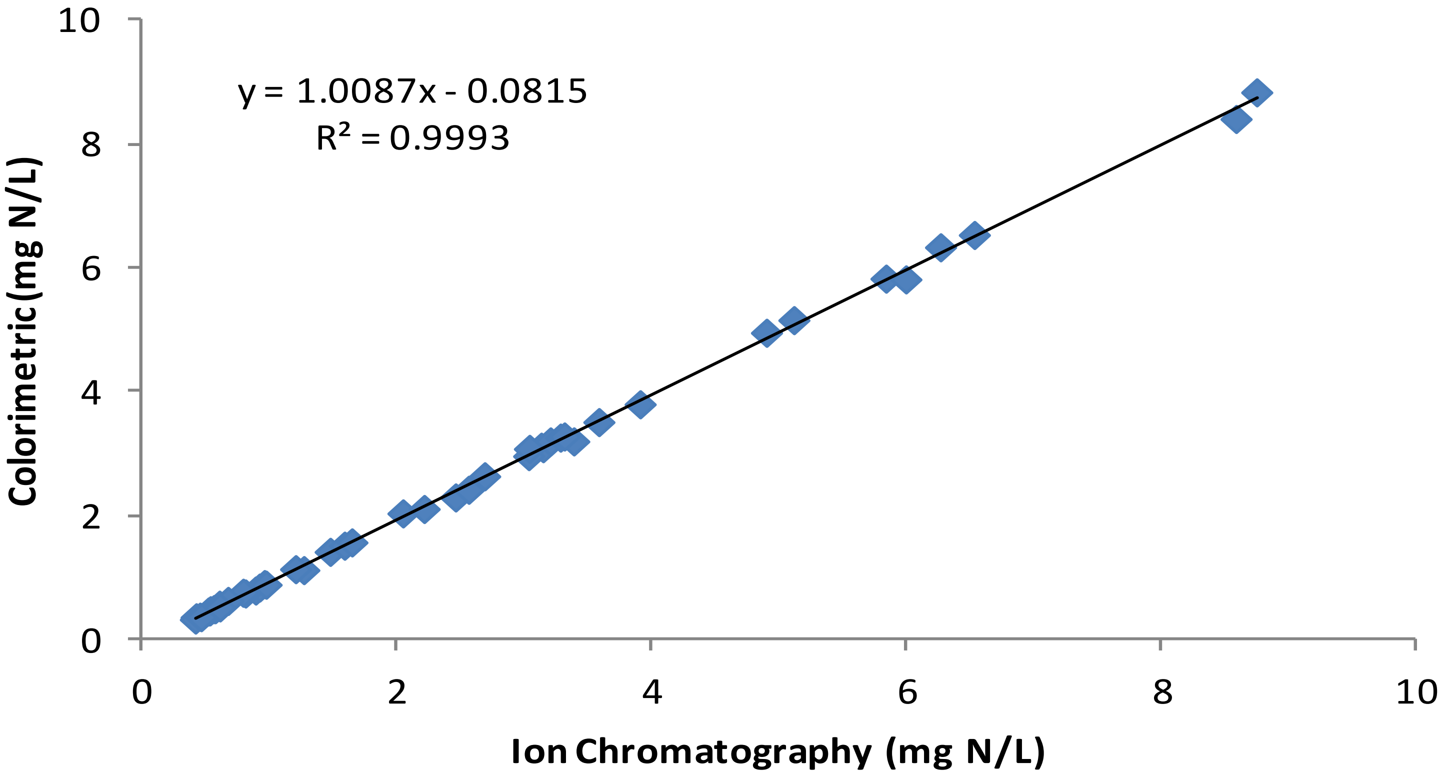
Sample[1]	Digested (mg N/L)	Undigested (mg N/L)	Calculated icTKN (mg N/L
SVL Effluent T3	8.54	6.52	2.02
SVL Primary Effluent	36.52	0.31	36.21
SJC Filtered Effluent	13.01	11.02	12.99
SJC Final Effluent	12.66	14.77	0
SJC TPS	13.43	11.69	1.74
SJC Raw Sewage	39.87	0.55	39.32

All results are calculated after accounting for the dilution factor

[1]SVL = Sunnyvale, CA; SJC = San Jose, CA

Comparison of Total N in River Water samplers from Alkaline Persulfate Digested samples using Ion Chromatography and Colorimetric Determinative Procedures

SM4500-N-C



ASTM D8001

Comparison of Total P in River Water samplers from Alkaline Persulfate Digested samples using Ion Chromatography and Colorimetric Determinative Procedures

TP (mg/L)	IC (mg/L)	%Difference
0.124	0.128	3.17
0.154	0.162	5.06
0.29	0.362	21.0
0.449	0.662	38.0
0.108	0.101	6.69

Influence of High Chloride Concentrations on the Recovery of N and P

Injected Chloride Concn (mg/L)	Test Compound	N or P Retention Time (min)	Nominal Concn (mg N or P/L)	Dilution	% Recovery
560	Glycine-N	9.10	1.49	18	104.5
	Glycerophosphate-P	16.8	1.63	18	96.2
997	Glycine-N	8.90	1.49	10	101.2
	Glycerophosphate-P	16.7	1.63	10	94.0
1472	Glycine-N	8.70	1.49	7	87.5
	Glycerophosphate-P	16.7	1.63	7	88.9

Next Steps: Multilab Validation

- EPA does not a method for Total N, goal is to allow use of D8001 for TKN. If approved, Subtract IC results for NO_3 Digested – undigested

icTKN = Digested – Undigested Sample

$$icTKN = TN - [(NO_3 - N) + (NO_2 - N)]$$

icTKN = D8001 – D4327 or EPA 300.1

- Requirements:
- Holding Time Study
  - 28 day vs. 48 hr.

Holding Time Regulations

- 40 CFR part 136.3(e) states: "Information in the table takes precedence over information in specific methods or elsewhere."
- *Discussion on icTKN vs. TKN*: should holding times be the same or 48 hr.?
  - Preservation by Acid addition would overload an IC column
  - However, parameters we are measuring have a 48 hr. holding time

Parameter	Preservation Requirements (40 CFR Part 136.3, Table II)	Maximum Holding Time (40 CFR Part 136.3, Table II)
Nitrate	Cool to ≤ 6º C	48 hours
Nitrite	Cool to ≤ 6º C	48 hours
Nitrate + Nitrite	Cool to ≤ 6º C , H_2SO_4 to pH <2	28 days
Total Kjeldahl Nitrogen (TKN)	Cool to ≤ 6º C , H_2SO_4 to pH <2	28 days
Orthophosphate	Cool to ≤ 6º C	48 hours*
Total Phosphorus	Cool to ≤ 6º C , H_2SO_4 to pH <2	28 days

EPA Alternative Test Procedure Requirements

- 9 matrices
- M/MS/MSD
- Proficiency Test sample
- MDL
- Blank
- Standards

Matrices	
1	ASTM Waste water
2	Wyoming POTW Effluent 1
3	Wyoming POTW Effluent 2
4	Wyoming POTW Influent
5	An Industry process water (explosives)
6	MWMP (Meteoric Water Mobility Procedure)
7	SPLP leachate
8	Pulp and Paper
9	Oil and Gas

- Would you like to participate in a Round Robin Study?

Conclusions

- Confirmed literature for the use of alkaline persulfate digestion with IC
- Confirmed with the use of hydroxide eluent
- Simplify method – digestion and analysis time
- Blank subtraction? Or use a higher MDL
- Improve MDL
- Determine interference levels – TOC and TDS

- Next Steps
- Multilab Validation

- THANK YOU!