

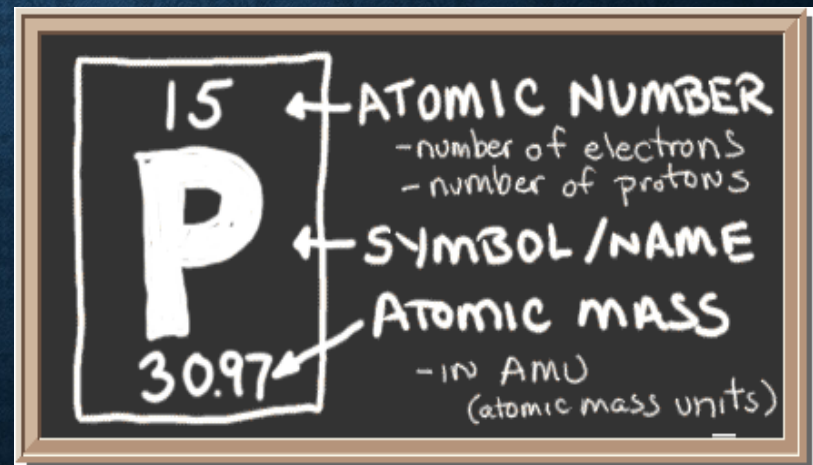
TOTAL PHOSPHORUS

**METHODS OF ANALYSIS, WASTEWATER
TREATMENT AND EFFECTS ON NATURE**

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NEORSD**

WHAT IS PHOSPHORUS?

- Phosphorus (P) is a nutrient that is vital to human, animal, and plant growth.
- It's one of the most common substances found in nature.
- It's found in our water, our food, and our bodies.



WHAT IS PHOSPHORUS?

- It's a highly reactive, nonmetallic element existing in three allotropic forms, white (or sometimes yellow), red, and black. In its pure form, it is toxic.



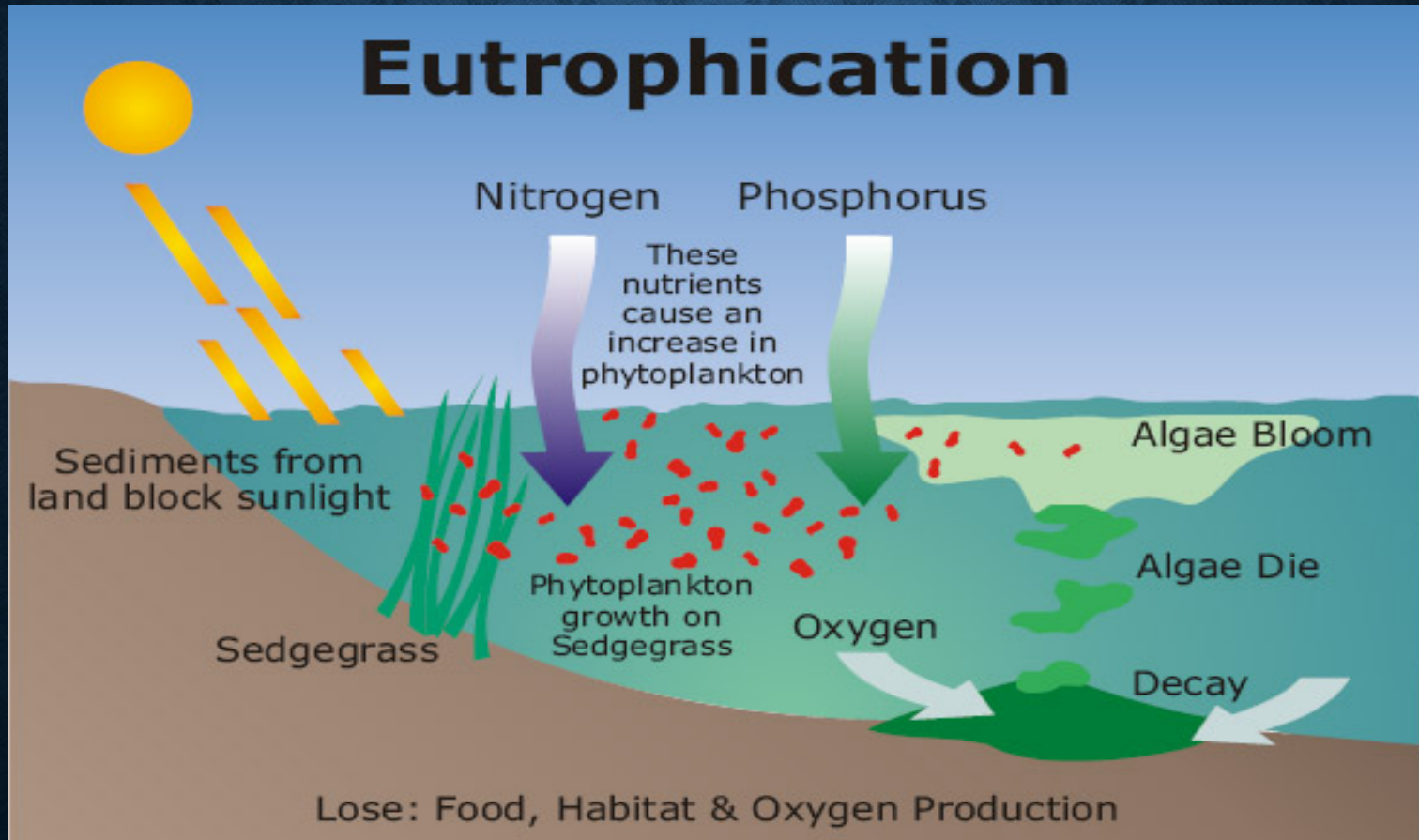
WHERE DOES IT COME FROM?

- Phosphorus occurs *naturally* at low levels in water, plants, and animals.
- Phosphorus occurs *unnaturally* in fertilizers (used in agriculture), cleaners (used in industry) and wastewater (from household sewage).
- Phosphorus is found in water, solids (detritus), and in the bodies of biological organisms.

SO WHY IS IT IMPORTANT?

- We need it to survive in low levels!
- However, high levels of Phosphorus in waterways causes eutrophication, an excess richness of nutrients, which leads to *algal blooms* and/or dense plant growth.
- This process decreases sunlight and oxygen levels (hypoxia) thus affecting fish and other aquatic life.

SO WHY IS IT IMPORTANT?



ALGAE – LAKE ERIE



ALGAL BLOOM - POND



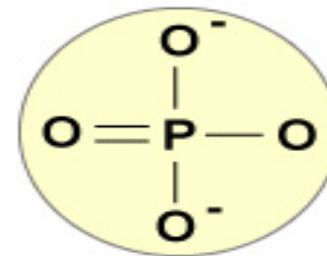
FORMS OF PHOSPHORUS

- Phosphorus (P) occurs
Phosphates (PO₄).

- These are classified as

Orthophosphates

(reactive phosphates), *Condensed Phosphates* (pyro, meta, and polyphosphates) and *Organic Phosphates*.



Phosphate
group

ORTHOPHOSPHATES

- Orthophosphates, also known as Reactive Phosphates, are a main constituent in fertilizers used for agriculture and residential purposes.
- Orthophosphates can be carried into streams and lakes through run-off.

CONDENSED PHOSPHATES

- Condensed (inorganic) phosphates are phosphorus compounds that contain salts and/or metals such as sodium, potassium, and calcium in various structures and chains.
- Condensed phosphates are used in industry and as food additives.



DID YOU KNOW?

- Phosphoric acid was (and still is in dark varieties) added to pop to enhance flavor and fizz.
- However, phosphoric acid can leach calcium from bones and teeth.
- High levels of phosphorus in the body can cause premature aging and cancer.

ORGANIC PHOSPHATES

- Organic Phosphates are formed primarily by biological processes (ex: *ATP – Adenosine Triphosphate*). ATP is a chemical compound that breaks down to release energy in the body.
- Organic phosphates enter sewage via human waste and food residues.
- Organic phosphates can be formed from orthophosphates in biological treatment processes or by receiving water biota.

PHOSPHORUS ANALYSIS

- Collection, Preservation and Holding Times
- Ortho vs Total Phosphorus
- Dissolved Phosphorus
- Different Methods of Analysis
- Digestion of Total Phosphorus
- Quality Control (QC) samples
- Interferences

COLLECTION AND PRESERVATION

- Collection: Plastic or glass
- Hold Time: Total Phosphorus: 28 days
Orthophosphate: 48 hours
- Preservation: Total Phosphorus: pH<2 H₂SO₄
Orthophosphate: No Preservation
- Storage: Refrigerate, <4°C

ORTHOPHOSPHATE VS. TOTAL PHOSPHORUS

- *Orthophosphate* (reactive) is analyzed directly on an unpreserved sample within 48 hours of sampling.
- *Total Phosphorus* (all forms) is analyzed on an acid preserved sample within 28 days of sampling following an acid digestion.

DISSOLVED PHOSPHORUS

- *Dissolved Reactive Phosphorus* samples are field filtered, within 15 minutes of sampling, through a 0.45- μm filter and analyzed within 48 hours.
- *Low Level Dissolved Reactive Phosphorus* samples are treated the same as above, except the filter size is 0.2- μm .
- *Dissolved Total Phosphorus* samples are filtered through a 0.45- μm filter prior to preservation. The sample is then digested and analyzed within 28 days of sampling.

PHOSPHORUS METHODS

- Colorimetric EPA Method 365.2 – Manual Spectrophotometer
- Semi-Automated Colorimetric EPA Method 365.1 – Automated Spectrophotometer (Flow injection Analysis –FIA)
- Standard Methods 4500-P
- Ion Chromatography (IC) EPA Method 300.0
- Discrete Analyzers

DISCRETE ANALYZER

- A instrument that employs robotics and syringes to aspirate, dispense, and mix appropriate amounts of sample and reagents into reaction wells.
- The sample/reagent mixture then incubates in the reaction well for a pre-programmed time.
- A single aliquot of the mixture is then transferred to a cuvette for spectrophotometric analysis.
- Absorbance is plotted against ppm in a linear regression curve to calculate concentration.
- Can analyze the various forms of phosphorus.

DISCRETE ANALYZER

- Advantages:
 - Reduces manual labor
 - Reduces reagents
 - Reduces sample volume
 - Reduces waste
- Disadvantages:
 - Instrument cost

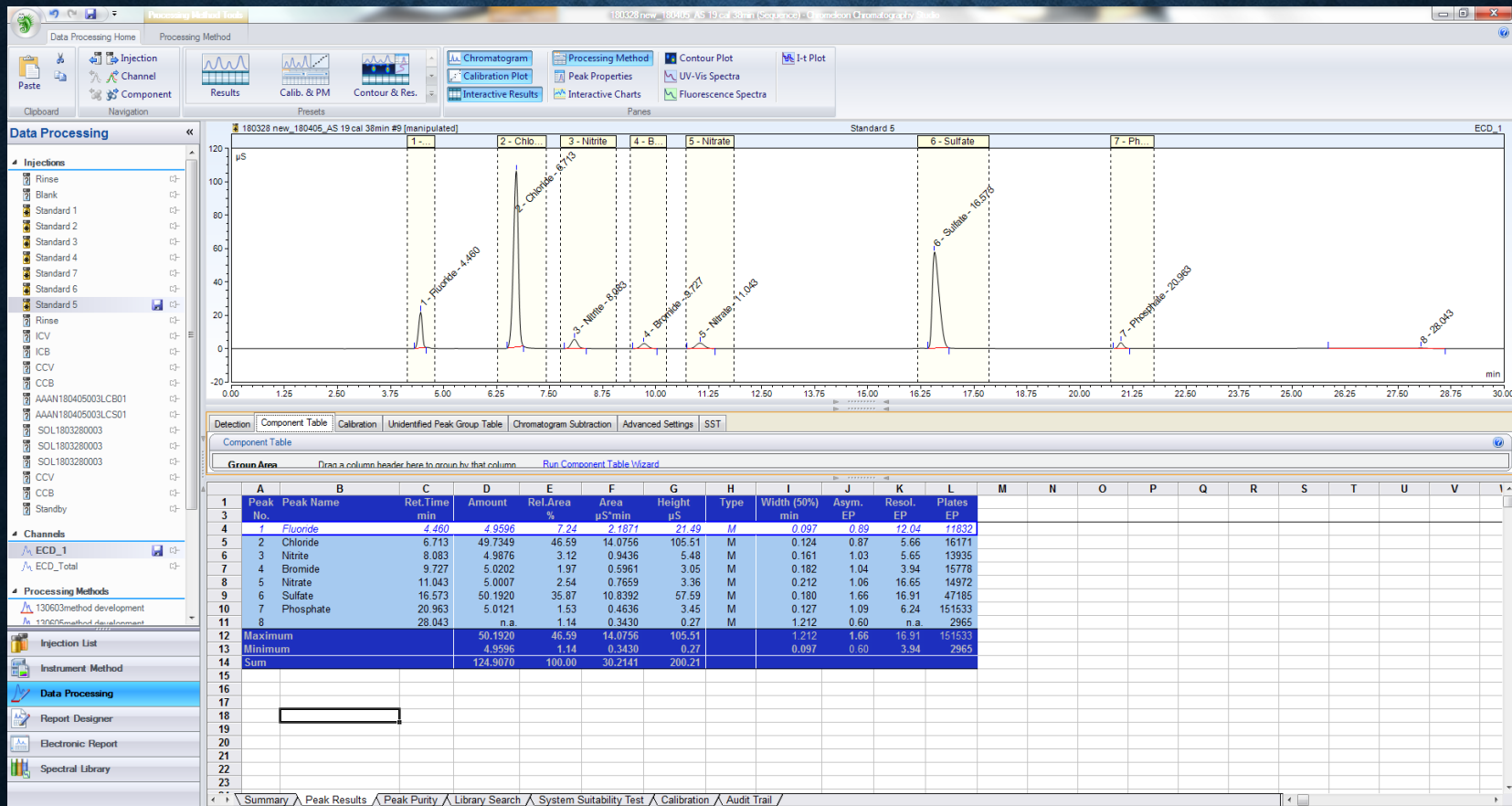
ION CHROMATOGRAPHY

- Anions are separated based on their affinities toward the stationary phase in the column. A suppressor cartridge chemically suppresses the background conductance of the eluent (dilute KOH solution) and converts the anions into species of higher conductance.

ION CHROMATOGRAPHY

- Using pressure and eluent, the sample is “pushed” through a stationary phase column. The anions are separated by size and then measured using a conductivity detector.
- The electronic signal is converted to peak area. Concentration is determined using a linear regression curve (plotting area vs. ppm).

ION CHROMATOGRAPHY



ION CHROMATOGRAPHY

- Analyzes Reactive (Ortho) Phosphate only
- Linear Range 0.25- 5.0mg/L for Phosphate
- Calibrate monthly
- Minimum of 4 Standards covering the linear range, from which a linear regression curve is created.
- ≥ 0.995 Correlation Coefficient
- Method Detection Limits (MDL) performed following new MDL rules with quarterly checks

LINEAR REGRESSION CURVE ION CHROMATOGRAPHY

A23		Standard 1							
	A	B	C	D	E	F	G	H	I
6									
7	Calibration Summary								
8	Peak Name	Eval.Type	Cal.Type	Points	Offset (C0)	Slope (C1)	Curve (C2)	Coeff.Det. %	
10	Fluoride	Area	Lin, WithOffset, 1/A	5.000	-0.003	0.442	0.000	99.8606	
11	Chloride	Area	Lin, WithOffset	5.000	-0.176	0.287	0.000	99.9959	
12	Nitrite	Height	Lin, WithOffset	5.000	0.035	1.092	0.000	99.9531	
13	Bromide	Area	Lin, WithOffset, 1/A	4.000	-0.006	0.120	0.000	99.9330	
14	Nitrate	Height	Lin, WithOffset, 1/A	4.000	-0.041	0.681	0.000	99.9985	
15	Sulfate	Area	Lin, WithOffset	5.000	-0.009	0.216	0.000	99.9990	
16	Phosphate	Height	Lin, WithOffset, 1/A	4.000	-0.030	0.694	0.000	99.9463	
18									
19	Injection Name	Amnt.Dev. %	Area $\mu\text{S}^*\text{min}$	Height μS	Amount				
20									
21	Fluoride	Fluoride	Fluoride	Fluoride	Fluoride				
22		ECD_1	ECD_1	ECD_1	ECD_1				
23	Standard 1	2.1937	0.0418	0.499	0.102				
24	Standard 2	-3.3326	0.1034	1.225	0.242				
25	Standard 3	-5.4123	0.2056	2.357	0.473				
26	Standard 4	7.3591	0.4708	5.116	1.074				
27	Standard 7	n.a.	8.2045	60.134	18.585				
28	Standard 6	n.a.	4.2537	37.186	9.639				
29	Standard 5	-0.8078	2.1871	21.494	4.960				
30									

IC ANALYSIS QC

- Initial Calibration Verification (**ICV**) and Initial Calibration Blank (**ICB**) analyzed daily
- Continuing Calibration Verification (**CCV**) and Continuing Calibration Blank (**CCB**) analyzed initially before samples, after every 10 samples and again at the end of the sequence
- Reporting Limit Check (**RLC**) analyzed with each batch containing Drinking Water samples

IC BATCH QC

- Laboratory Control Blank (**LCB**) and Laboratory Control Sample (**LCS**) prepared fresh daily per batch of **20** samples
- Duplicate/Matrix Spike or MS/MSD per **10** samples. Percent Recovery and Relative Percent Difference (RPD) are calculated

IC SAMPLE PREPARATION

- All samples and batch QC (LCB, LCS, Duplicates, MS/MSD) are filtered through a 0.20-mm syringe filter to remove all particulate.
- MS/MSD's are spiked as to be within the analysis range.

IC INTERFERENCES

- All solids must be filtered out. Any particulate in the system will cause problems and plug up the system.
- High concentrations of some ions will cause false readings of other ions.
- Peak shifting: as the column gets older, peaks may shift causing misidentification.

IC

ADVANTAGES/DISADVANTAGES

- Analyzes up to 7 Anions in less than 15 minutes
- Can be very stable
- Can run overnight (load and go!)
- No hazardous waste
- Small sample volume needed
- May have to do multiple dilutions per sample
- Each injection takes up to 30 minutes to run

PHOSPHORUS 365.1/365.2

- Phosphorus is analyzed using colorimetric (spectrophotometric) analysis. This can be performed manually or automated.
- *Orthophosphate (Reactive Phosphorus)* is analyzed directly.
- *Total Phosphorus (as P)* is analyzed after going through an acid digestion to convert all forms of phosphorus to orthophosphate.

METHOD SUMMARY

- Ammonium molybdate and antimony potassium tartrate react in an acid medium with orthophosphate to form a phosphomolybdic acid, which is then reduced to an intense molybdenum blue color by ascorbic acid. The resultant color is read spectrophotometrically at 880-nm.
- This can be performed on a manual spectrophotometer or on an automated FIA spectrophotometer.

ORTHOPHOSPHATE (REACTIVE)

- Orthophosphate (reactive) is analyzed directly on an unpreserved sample within 48 hours of sampling.
- No preparation needed for Manual Spectrophotometer
- For Automated Spectrophotometer, samples must be filtered through Whatman 41 filter paper to remove solids
- Dissolved Reactive Phosphorus and Low Level Dissolved Reactive Phosphorus filtered through 0.45-mm and 0.2-mm syringe filter, respectively, IN THE FIELD

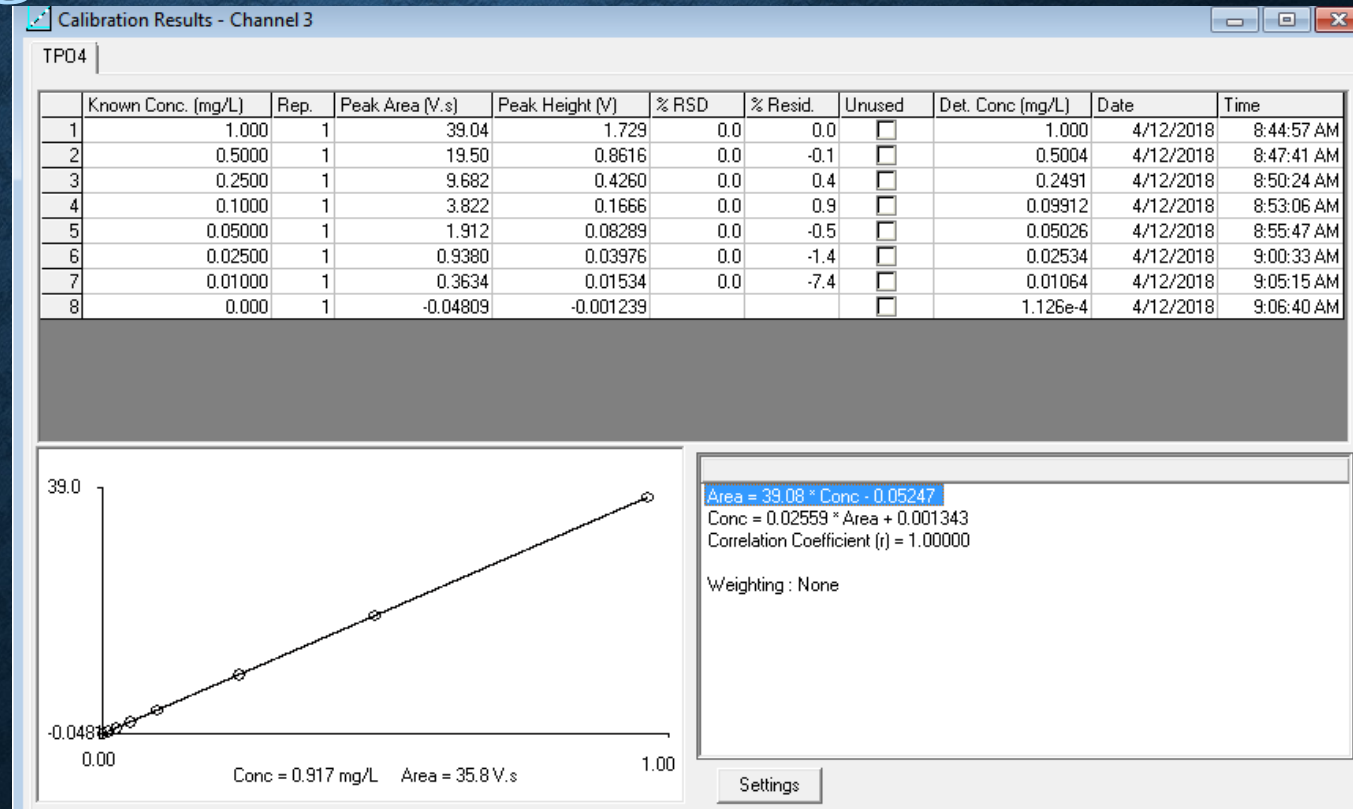
ORTHOPHOSPHATE (REACTIVE)

- Calibrate Daily
- Linear Range 0.01-2 mg/L
- Minimum of 5 Standards covering the linear range, from which a linear regression is created.
- ≥0.995 Correlation Coefficient
- Method Detection Limits (MDL) performed following new MDL rules with quarterly checks.

ORTHOPHOSPHATE

- Final results are obtained from the linear regression curve created with the known standard concentrations.

Linear Regression Curve



ANALYSIS QC

- Initial Calibration Verification (**ICV**) and Initial Calibration Blank (**ICB**) analyzed daily
- Continuing Calibration Verification (**CCV**) and Continuing Calibration Blank (**CCB**) analyzed initially before samples, after every 10 samples and again at the end of the sequence

BATCH QC

- Laboratory Control Blank (**LCB**) and Laboratory Control Sample (**LCS**) prepared fresh daily per batch of **20** samples
- Duplicate/Matrix Spike or MS/MSD per **10** samples. Percent Recovery and Relative Percent Difference (RPD) are calculated

TOTAL PHOSPHORUS

- **Method Summary: Total Phosphorus** analysis has two procedural steps:
 - *ONE* - convert all phosphorus forms to orthophosphate using acid hydrolysis.
 - *TWO* - analyze orthophosphate colorimetrically, either manually or automated.

TOTAL PHOSPHORUS DIGESTION

- In the *acid hydrolysis step*, sulfuric acid and ammonium persulfate (peroxydisulfate) are added to a known volume of sample. It is then digested on a hotplate to near dryness. The sample is cooled and diluted back to the original volume. Sample is then filtered to remove any solids for Auto-Analysis.
- Alternatively, an autoclave may be used for digestion (30 min at 121°C, 15-20 psi) or hotblock. Both are a closed digestion, therefore no evaporation nor dilution required (30 min at 150°C).

TOTAL PHOSPHORUS ANALYSIS

- The *colorimetric step*, is the same as the orthophosphate in that, Ammonium molybdate and antimony potassium tartrate react in a acid medium with orthophosphate to form a phosphomolybdic acid, which is then reduced to an intense molybdenum blue color by ascorbic acid. The resultant color and read spectrophotometrically at 880-nm
- The colorimetric step may be performed on a manual spectrophotometer or on an automated analyzer.

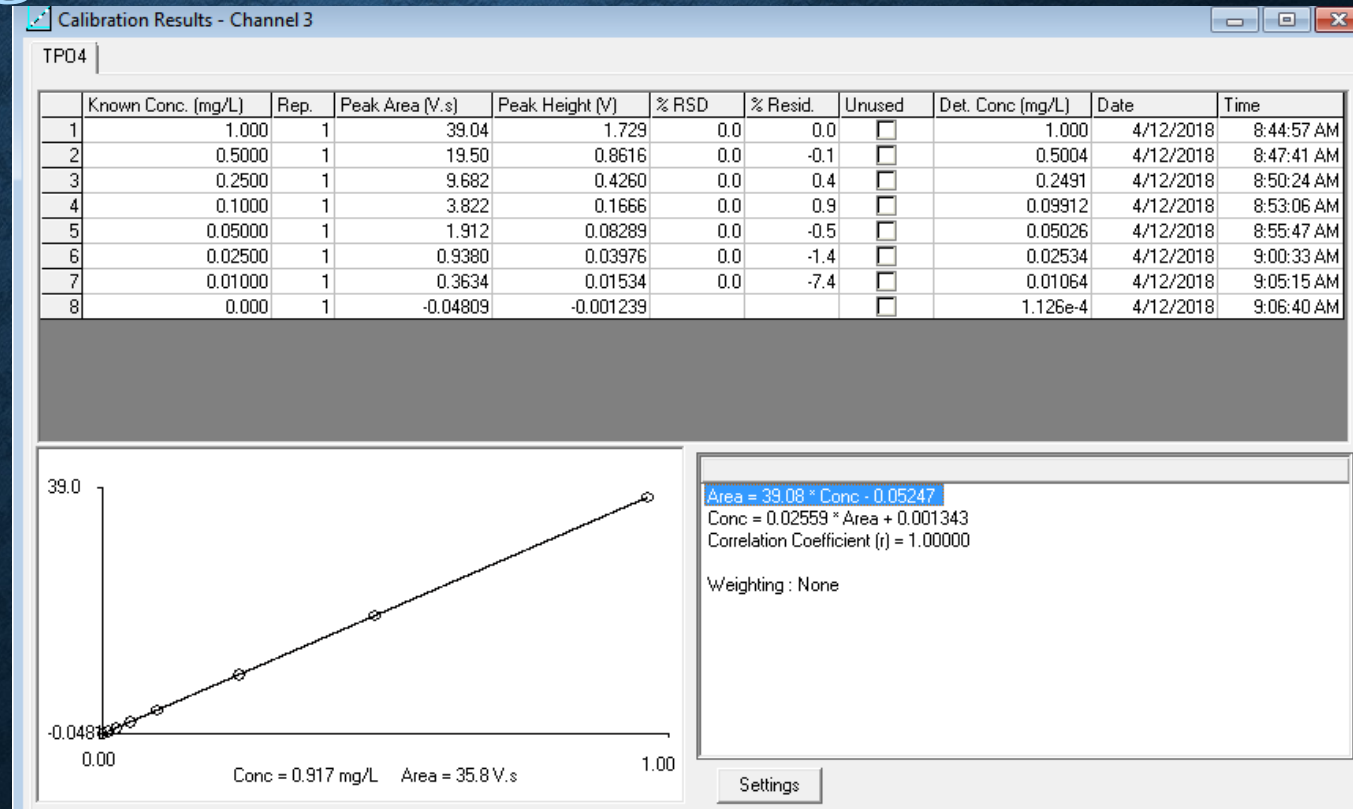
TOTAL PHOSPHORUS ANALYSIS

- Calibrate Daily
- Linear Range 0.01-2 mg/L (ours 0.02-1 mg/L)
- Minimum of 5 Standards covering the linear range from which a linear regression is created.
- ≥ 0.995 Correlation Coefficient
- Method Detection Limits (MDL) performed following new MDL rules with quarterly checks

TOTAL PHOSPHORUS

- Final results are obtained from the linear regression curve created with the known standard concentrations.

Linear Regression Curve



ANALYSIS QC

- Initial Calibration Verification (**ICV**) and Initial Calibration Blank (**ICB**) analyzed daily
- Continuing Calibration Verification (**CCV**) and Continuing Calibration Blank (**CCB**) analyzed initially before samples, after every 10 samples and again at the end of the sequence

BATCH QC

- Laboratory Control Blank (**LCB**) and Laboratory Control Sample (**LCS**) digested fresh daily per batch of **20** samples
- Duplicate/Matrix Spike or MS/MSD per every **10** samples. Percent Recovery and Relative Percent Difference (RPD) are calculated

QC FAILURE & CORRECTIVE ACTIONS

- Calibration Criteria, such as residuals or correlation coefficient out of range, results in recalibrating the instrument.
- Analysis QC criteria not met, results in re-analyzing the initial or continuing QC. Repeated failure results in recalibrating the instrument.
- Batch QC results out of acceptable range may result in re-digestion and/or re-analysis of QC and samples.

PHOSPHORUS INTERFERENCES

- As with any colorimetric analysis, *turbidity* (solids) can be a positive interference but can be removed with filtration. Filtration must occur prior to analysis, not prior to digestion (unless the sample is dissolved) on total phosphorus samples.
- Sample color may also interfere. Background correction can be performed in this case.

PHOSPHORUS INTERFERENCES

- High levels of arsenic can cause a positive interference if the As concentrations are higher than the phosphorus concentration.
- High levels of iron may cause the precipitation of, and subsequently the loss of phosphorus.

TROUBLESHOOTING

- Contamination!! Contamination!! Contamination!!
- All glassware must be washed with hot water and phosphate free soap, rinsed with dilute hydrochloric acid and rinsed with copious amounts of de-ionized water.
- All instrument components and reagents must be phosphorus free.

TROUBLESHOOTING

- FIA Auto-Spectrophotometers: *Plugs* and *leaks* are the most common problems.
- Routine Maintenance helps prevent problems:
 - Changing pump and tygon tubing
 - Rinsing and/or cleaning manifold tubing
 - Keeping area clean and dust free
 - Keeping reagents covered when in use

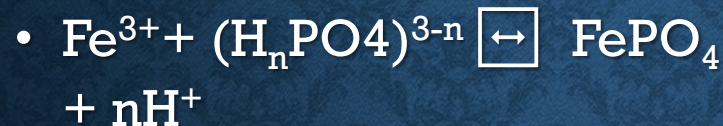
TREATMENT OF PHOSPHORUS

- Some of the Phosphorus entering the treatment plant is removed by primary (settling) and secondary treatment. However, phosphorus remains in the solids.
- Additional phosphorus is removed by adding Ferric Chloride, Ferrous Sulfate, Alum, or Lime during the waste treatment process.

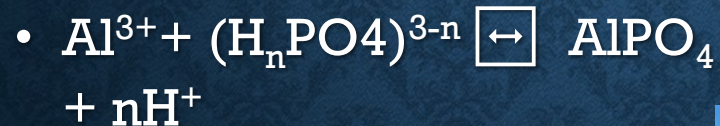
TREATMENT OF PHOSPHORUS

- Chemical removal

- Ferric Chloride



- Aluminum Sulfate (Alum)



PHOSPHORUS

Any Questions??

PHOSPHORUS

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