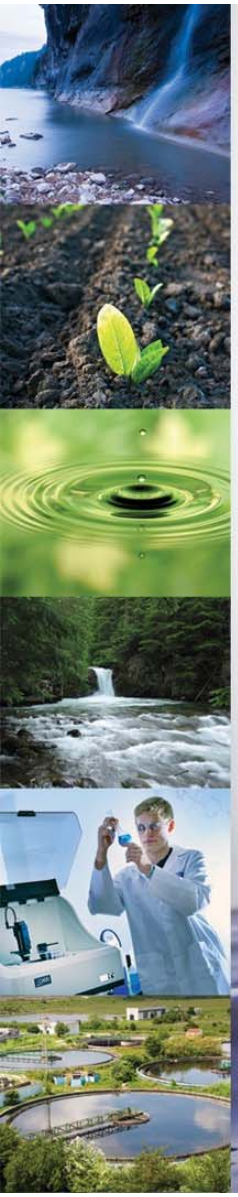

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World Leader in Sample Preparation, Segmented Flow and Discrete Analyzer Technology

Troubleshooting for Total Phosphorous and Ortho-Phosphate Colorimetric Testing for Discrete and Segmented Flow Analyzers

Sarah Leibenguth

Technical Sales & Support Chemist

Topics for Discussion

- Method Principle
- Test Procedures
- Digestion Protocol
  - Digestion Reagents
  - Digestion pH
  - Temperature
  - Digestion Block
  - Reconstitution

Topics for Discussion

- Colorimetric Detection
  - Reagents
  - Interferences
  - Riley Ratio
  - pH and Matrix Matching

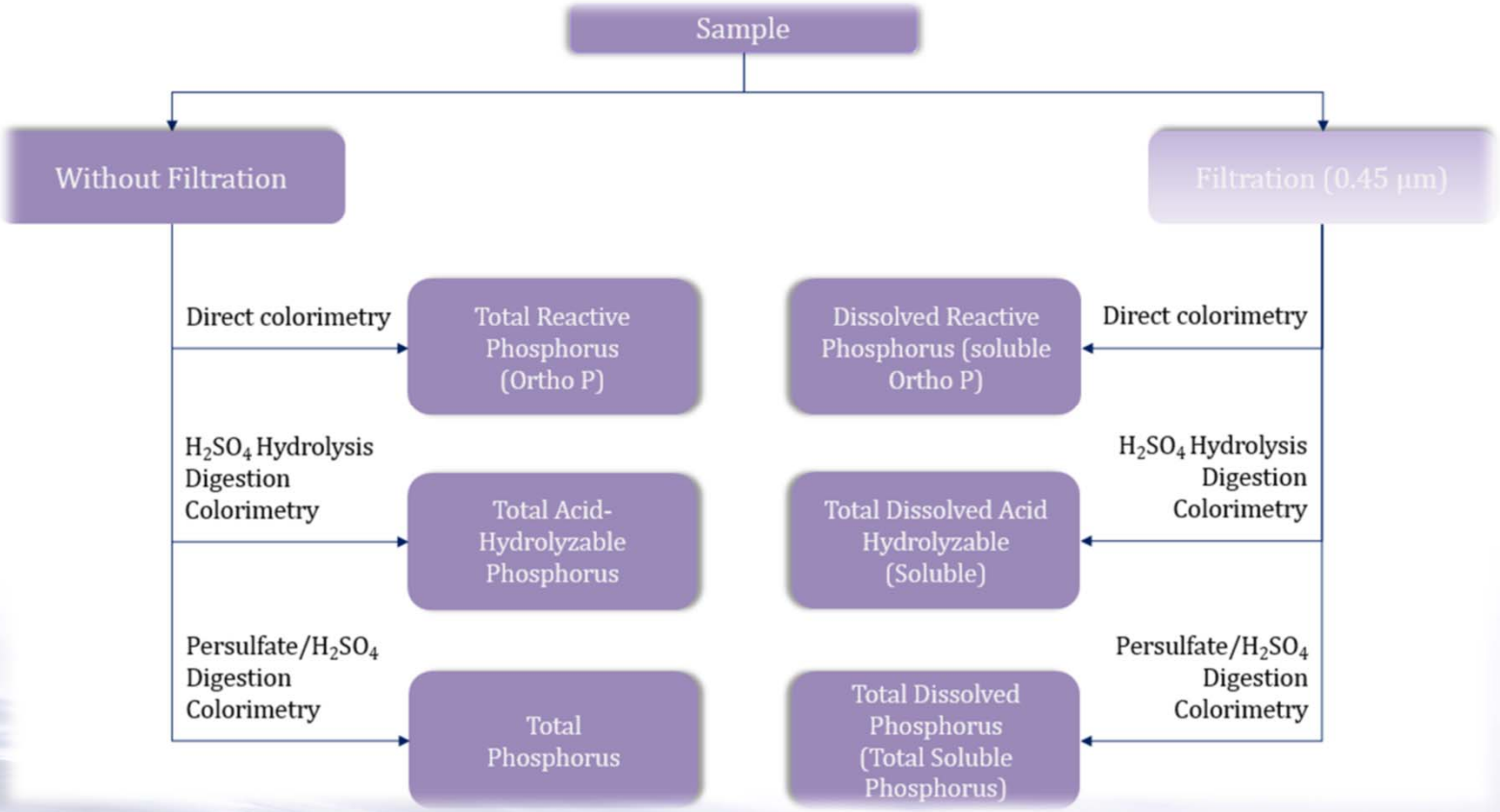
Method Principle and Approval

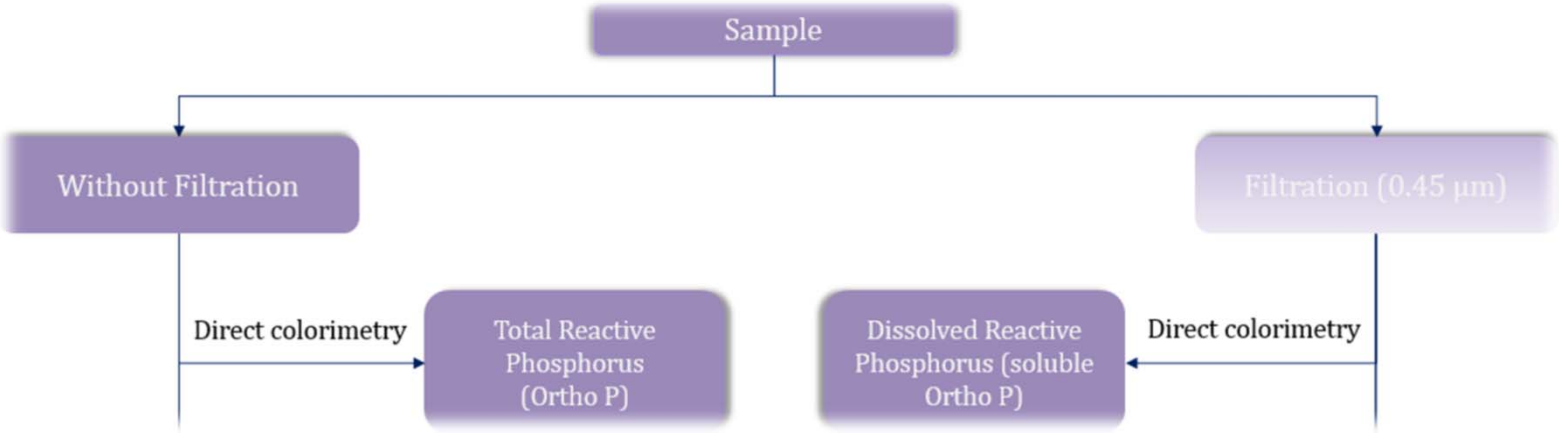
Method Principle

- TP and oP Colorimetric Reaction
- Ammonium Molybdate
  - Orthophosphate + Molybdate → Phosphomolybdic Acid
- Antimony Potassium Tartrate
  - Catalyst for Phosphomolybdic Acid
- Ascorbic Acid
  - Phosphomolybdic Acid + Ascorbic Acid → Blue Phosphomolybdic Complex

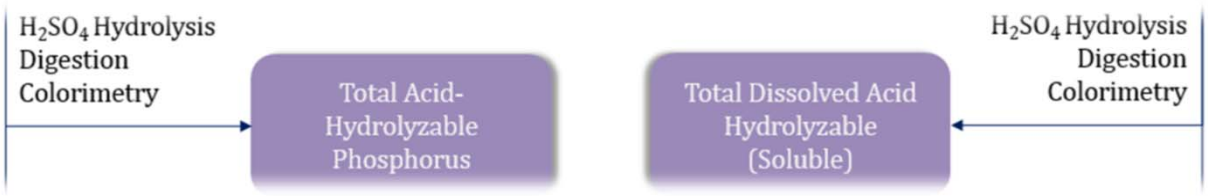
Test Procedures

- Ortho-Phosphate
  - EPA 365.1, version 2 (1993)
  - Standard Methods 4500-P F (18^{th},19^{th},20^{th})
- Total Phosphorous
  - EPA 365.1, version 2 (1993)
  - Standard Methods 4500-P B, F (18^{th},19^{th},20^{th})
- Total Kjeldahl Phosphorous
  - EPA 365.4


Sample
Without Filtration
Filtration (0.45 µm)
Direct colorimetry
Total Reactive Phosphorus (Ortho P)
Dissolved Reactive Phosphorus (soluble Ortho P)
Direct colorimetry
H_2SO_4 Hydrolysis Digestion Colorimetry
Total Acid-Hydrolyzable Phosphorus
Total Dissolved Acid Hydrolyzable (Soluble)
H_2SO_4 Hydrolysis Digestion Colorimetry
Persulfate/H_2SO_4 Digestion Colorimetry
Total Phosphorus
Total Dissolved Phosphorus (Total Soluble Phosphorus)
Persulfate/H_2SO_4 Digestion Colorimetry



- Reactive Phosphorous
  - Available oP for Colorimetric Detection
    - Prior to Hydrolysis or Digestion
    - Filtered or Non-Filtered Samples
    - Total Reactive or Dissolved Reactive



- Acid-Hydrolyzable Phosphorous
  - Pre-Treated by Acid Hydrolysis at Boiling Temperature
    - Filtered or Non-Filtered Samples
    - Total Acid-Hydrolyzable or Total Dissolved Acid-Hydrolyzable

- Total Phosphorous
  - Digestion Pre-Treatment
    - Organically Bound Phosphorous Converted to oP
    - Filtered or Non-Filtered Samples
    - Total Phosphorous or Total Dissolved/Soluble Phosphorous

Total Phosphorous Digestion Protocol

Total Phosphorous Digestion

- Persulfate Digestion
  - Hot Plate or Block Digestor
  - Autoclave
- Kjeldahl Digestion
  - High Temperature Block Digestor

Total Phosphorous Persulfate Digestion

TP Persulfate Digestion

- Persulfate Digestion Reagent
  - Potassium Persulfate
  - Ammonium Persulfate
    - Easier to Dissolve
  - Sulfuric Acid
  - Prepare Combined Reagent Immediately Prior to Use
- Digestion pH
  - Persulfate Concentration
  - Acidic Digestion
  - Alkaline Digestion

TP Persulfate Digestion

- Temperature
  - Hot Plate or Block Digestor
    - Boil 30 min or 5 -10 mL Final Volume
    - Cool and Reconstitute
    - Block Stability
  - Autoclave
    - Approximately 120°C
    - Heat for 30 min
- Reconstitution
  - Not Necessary for Autoclave!
  - Accurate Volumes
  - Block Digestor Automation

Total Phosphorous Kjeldahl Digestion

TP Kjeldahl Digestion

- TKP Digestion Reagent
  - Sulfuric Acid
  - Potassium Sulfate
  - Heat to 380°C
  - Catalyst
- Digestion pH
  - Incomplete Digestions
  - Sample Acidity Level

TP Kjeldahl Digestion

- Evaporation Step in Protocol
  - Reduces Sample Volume Prior to Temperature Increase
  - Decreases Potential for Splatter
  - Final Volume Consistency
  - Example:

    Step to 160°C and Hold for 30 min
    Step to 190°C and Hold for 30 min
    Approximately 5 mL Final Volume
    Heat to 380°C and Hold for 30 min

Reconstitution

- Cooling
  - Acid Volume Remaining in Digestion Tube
  - DI Water Addition
  - Cool Digestion Tubes
  - Reconstitute
- Dispense Accurately
  - Bottle Top Dispenser or Auto-Pipette
  - Samples and Standards Same Final Volume
  - Volumetric Digestion Tubes
- Vortex Mixer

Catalysts

- Mercury
  - Documentation
  - Colorless Digest
  - Preparing Digestion Reagent
  - Toxic
  - Waste Disposal Considerations
- Copper
  - Substitute for Mercury Catalyst
  - Blue/Green Final Digest
  - Less Toxic

TP and Ortho-Phosphate Colorimetric Reaction

Colorimetric Reaction

- Reagents
  - Stability
  - Storage
  - Preparation
  - Additional Considerations

Reagents

- ACS Reagent Grade Chemicals
- Purifying System for Reagent Water
- Acid Rinse Reagent and Standard Containers

Reagents

- Ammonium Molybdate
  - Remake Monthly
  - Stored in Plastic Bottle
  - Store in the Refrigerator
  - Prone to Precipitate and Turbidity
  - Dry Chemical White in Appearance
  - No Green Coloration
  - Prepared Reagent is Colorless

Reagents

- Sulfuric Acid
  - Stored Indefinitely at Room Temperature
  - Cool Prior to Final Dilution
- Antimony Potassium Tartrate
  - Remake Monthly for Best Results
  - Stored in Glass Container
  - Store in the Refrigerator
  - Expired Reagent Concerns

Reagents

- Prepared Color Reagent
  - Add Reagents in Specific Order
    - Sulfuric Acid
    - Antimony Potassium Tartrate
    - Ammonium Molybdate
  - Turbidity
  - 1 – 3 Week Stability
  - Store in Refrigerator in Plastic Bottle
  - 4 Hour Stability if Ascorbic Acid Added

Reagents

- Ascorbic Acid Reagent
  - Stable 1 Week
  - Prepare Daily for Best Results
  - Stored in the Refrigerator
  - Discard if Yellow
  - Expired Reagent Concerns

Interferences

- Nitrite and Hexavalent Chromium
  - Low Concentration Readings
- Arsenate
  - Positive Interference
  - Similar Colorimetric Reaction
- Salt Concentrations
  - Less Than 1% Error for Salt Concentrations up to 20%(w/v)
- Turbidity
  - Filtration if Applicable

Interferences

- Iron, Copper, and Silica
  - High Silica Concentrations May Cause Positive Interference
  - 50 mg Fe^{3+}/L, 10 mg Cu/L and 10 mg SiO_2/L Tolerated
  - High Iron Concentrations May Cause Precipitation of Phosphorous

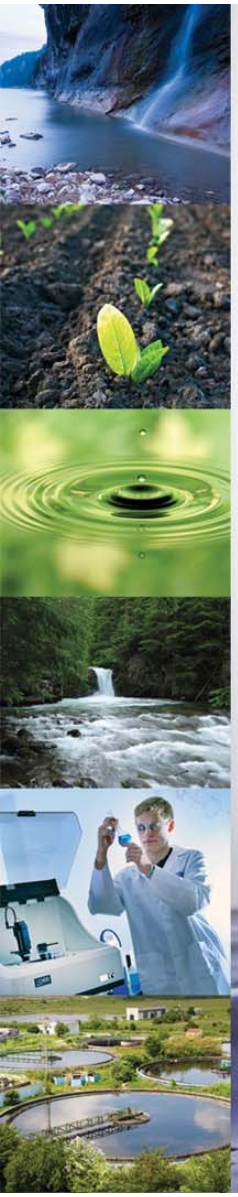
- Surfactants in SFA Reagents
  - SDS or Triton
  - No BRIJ!

Riley Ratio

- Acidic Environment Needed to Form Phosphomolybdic Acid
- The [H+] : [Mo] Ratio for Stable and Rapid Results
- Sample pH Changes Riley Ratio
- Final Sample Digest Changes Riley Ratio
- Method Adjustments for Acidified or Digested Samples

pH and Matrix Matching

- Sample Matrix and Carrier Solution
  - Acidified Samples
  - Saline Samples
  - Digested Samples
  - Standards and Control Treatment



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