Sampling and Sample Preservation How did we make something simple so complex? NEMC August 2019

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We are

One lab and two locations

Monrovia

South Bend

At the beginning of each EPA approved method, we are truly blessed

We have a section called sampling and sample preservation, however named

Sample Dechlorination and Preservation -- All samples should be iced or refrigerated at 4 C and kept in the dark from the time of collection until extraction. Residual chlorine should be reduced at the sampling site by addition of 40-50 mg of sodium sulfite (this may be added as a solid with stirring or shaking until dissolved) to each water sample. It is very important that the sample be dechlorinated prior to adding acid to lower the pH of the sample. Adding sodium sulfite and HCl to the sample bottles prior to shipping to the sampling site is not permitted. Hydrochloric acid should be used at the sampling site to retard the microbiological degradation of some analytes in water. The sample pH is adjusted to <2 with 6 N hydrochloric acid.

This is the same pH used in the extraction, and is required to support the recovery of acidic compounds like pentachlorophenol.

Text from Method 525.2

Where do statements like these come from? To highlight

- Refrigerated to 4 °C
- Kept in the dark
- Remove residual chlorine at time of sampling
- Adjust pH to < 2 with HCL
  - The same pH as the extraction? Really?
  - What does < 2 mean? 1?

Section 11.1.3 of method 525.2 says:

the pH of the sample should be **about 2**.

If residual chlorine is present and/or the pH is >2, the sample may be invalid.)

- Why invalid if pH > 2?
- Can I adjust to about 2?
- What compounds will I lose?
- Why does chlorine matter now?

But that's not all, Section 13.2.5 Method performance says:

The data in Tables 3, 4, 5, 6, and 8 are from samples **extracted at pH 2**.

Table 3 = P/A from reagent water using a C-18 cartridge and quadrupole
Table 4 = P/A from reagent water using a C-18 disk and quadrupole
Table 5 = P/A from reagent water using a C-18 cartridge and ion trap
Table 6 = P/A from reagent water using a C-18 disk and ion trap
Table 8 = P/A from reagent water using a C-18 cartridge and ion trap

The method validation data was extracted at pH 2

So to summarize, from method 525.2 we have:

- Preserve samples at pH < 2
- Extract samples at pH about 2, but if not pH 2 samples are no good
- Validation was done at pH 2
    - What does pH < 2 mean?
    - Why can't I adjust to pH 2?

Are there discrepancies in other methods?

40 CFR Part 136.3 Table II

48. Phenols .G .....Cool, ≤6 °C, H_2SO_4 to pH < 2

Method 420.1
Biological degradation is inhibited by the addition of 1 g/L of copper sulfate to the sample and acidification to a pH of less than 4 with phosphoric acid

ASTM D1783
Acidify the samples to a pH between 0.5 and 2.0 with H_3PO_4, HCl, H_2SO_4, or $NaHSO_4$.

Are there discrepancies in other methods?

40 CFR Part 136.3 Table II

Metals (200.7) P, FP, G ..... HNO3 to pH < 2

ASTM D1976 Research Report (same data as 200.7)

8.2.3 For the determination of total or total recoverable elements, the sample is acidified with (1+1) HN03 to pH 2 or less as soon as possible, preferably at the time of collection.

Proposed change to how we word preservation in methods

Draft ASTM Ammonia Method

10.2 Preserve samples by adding H_2SO_4 to pH 2 and storing at 2 to 6°C. Verify that pH is about 2 with 0 – 13 pH paper (or equivalent).

Note: Check residual chlorine using Starch Iodide paper. If positive, remove chlorine by adding 0.025 N Sodium Thiosulfate dropwise until there is no reaction with the starch paper..

Let's look at pH a little closer, do these "small differences" matter?

0	1 M	Battery Acid
1	0.1 M	Stomach Acid
2	0.01 M	Vinegar
3	0.001 M	Orange Juice

Does pH ~ 2 to pH < 2 (0 to 1) change the chemistry of the method?

How do you "verify" the pH of the sample? The methods don't say.

Using pH paper or pH strips to verify preservation of samples at login

pH paper ,n, -pieces of paper that change color depending on the pH.

pH strips, n – strips of plastic with one or more squares or rectangles of pH paper fastened to one end. Each square changes color depending on the pH

What exactly is pH paper?

pH paper is a cheap and relatively accurate way of **estimating** the pH of an aqueous liquid.

A strip of filter paper is soaked with different pH indicators, or a mixture of indicators (universal indicator), and allowed to dry.

Touching solution to the paper or dipping the paper in solution causes the color of the indicator on the paper to change according to the pH. The color and relative intensity of the color is compared to a chart from which pH is estimated.

What are pH strips?

pH strips consist of one or more (usually up to four) small squares or rectangles of pH paper attached to one end that is dipped into the sample.

Each square is impregnated with a different pH indicator resulting in different colors and intensity in relation to the pH.

The color and intensity of each square is compared to a chart from which pH is estimated.

The different indicators/colors of each square provide users with greater confidence in their estimation of the pH.

Universal instructions for such a universal test?

Explicit measurement instructions	Well defined immersion or reaction times	Well defined measurement practices
Dip	2 seconds	Compare to chart
Dip	20 seconds	Compare to chart
Dip	Till no color change	Compare to chart
Dip	1 – 10 minutes	Compare to chart

Accuracy of color is assumed
No variability of lighting in which observation is made?
No requirement to verify lots?

How does pH paper compare to pH using a meter?

F-Test Two-Sample for Variances	a	0.05			
	Paper	Meter	95% Confidence Interval		
Mean	4.60869565	4.26086957			
Variance	1.032	2.894	1.551	5.067	
Observations	46	46			
df	45	45			
F	2.80				
P(F<=f) one-tail	0.000	0.001	Two-tail		
F Critical one-tail	1.64				
One-tail	Reject Null Hypothesis because p < 0.05 (Variances are Different)				
Two-tail	Reject Null Hypothesis because p < 0.05 (Variances are Different)				

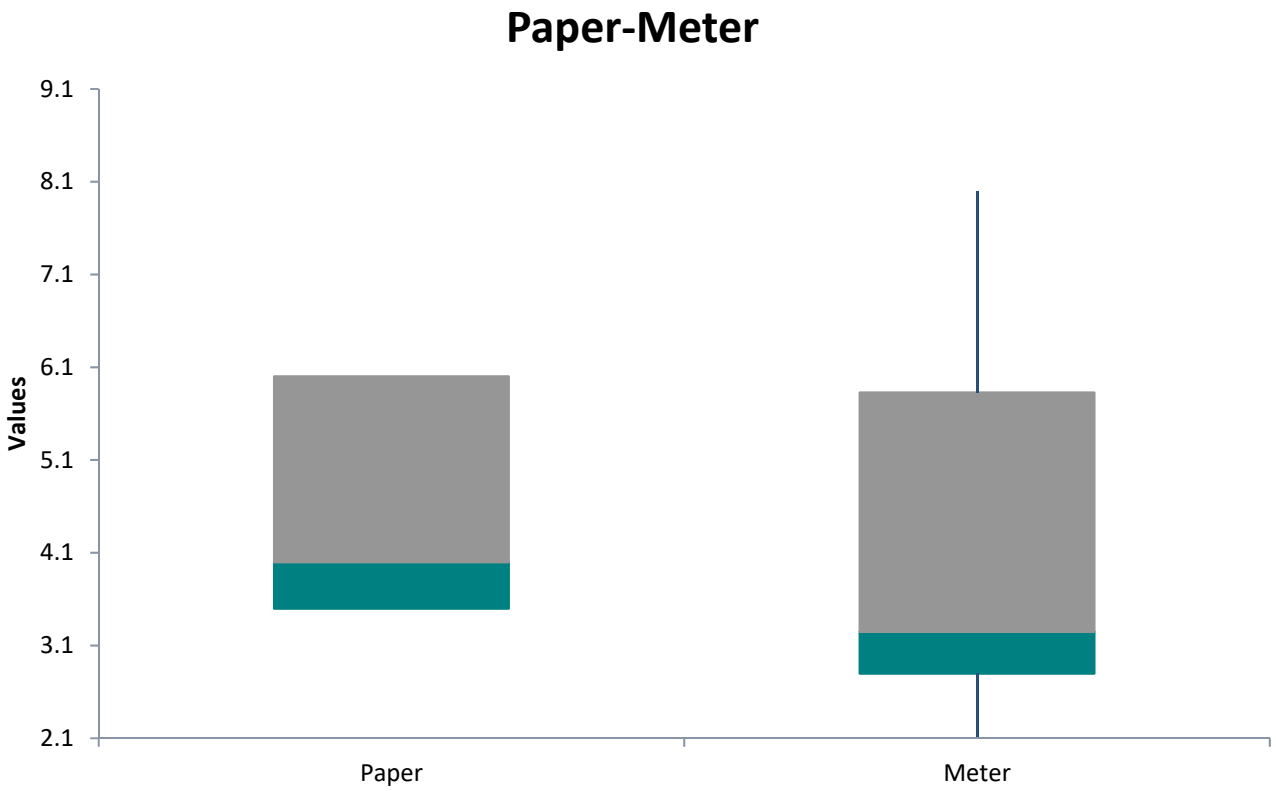
46 observations, over several days F Test Fails = Variance different

How does pH paper compare to pH using a meter?

Equivalence Test for Means	a	0.05			
Equal Sample Sizes					
	Paper	Meter			
Mean	4.60869565	4.26086957			
Variance	1.03236715	2.89443478			
Observations	46	46			
Pooled Variance	1.96340097				
Hypothesized Mean Difference	0				
df	90				
t Stat	1.190	-1.19047853			
	0.118	0.118	Cannot conclude means are equivalent		
T Critical one-tail	1.662				
P(T<=t) two-tail	0.237				
T Critical Two-tail	1.987				

46 observations, over several days Equivalence Test Fails = means are different

How does pH paper compare to pH using a meter?



Observations on verifying preservation of samples with pH paper or strips

- Bias of paper/strips about ± 0.7 for narrow range and ± 1 for wide range
- Paper is an INDICATOR and should not be perceived as a true value
- Comparison is arbitrary – train with buffers
- Paper varies by lot and within lot – verify color with buffers, or create your own comparison chart

Example Method 525.2 → pH 0 and 1 are < 2, but pH 4 low enough to extract analytes

Which chlorine do you check and how?

- Many methods just say chlorine. Is that “free” or total?
- They do not specify test (DPD, strips, amperometry?)
- Looksee or read it on a meter?
  - QA/QC for an out of hold screening test?

How does one measure “free” chlorine?

- DPD test not interference free
  - 5 ppm Chloramine can read up to 0.6 mg/L “free chlorine”
  - 1 ppm chloramine ~ 0.1 – 0.3 mg/L “free chlorine”

  - 0.1 – 0.3 mg/L “free chlorine” → samples rejected

- What does “immediately mean?

Case study, EPA method 552.3, section 8

Enough ammonium chloride must be added to the sample to convert the free chlorine residual in the sample matrix to combined chlorine.

Chloramines, formed by the reaction of hypochlorite with the ammonium ion, do not react further to produce additional haloacetic acids at significant concentrations and protect against microbiological degradation.

This concentration of ammonium chloride was determined to convert 8 mg/L of free chlorine residual to combined chlorine

Case study, EPA method 552.3, UCMR4 Table 13 sample acceptance criteria

Haloacetic acids ammonium chloride, 0.10 g/L Absence of free chlorine, less than 0.1 mg/L

7.3.3 The verification of the absence of free chlorine is qualitative, provided the laboratory can measure the presence of free chlorine at and above 0.1 mg/L.

When a positive free chlorine result occurs, recheck with a test strip of a second method, **such as the free chlorine DPD method.**

Two labs, two ways to screen for free chlorine

Lab 1	Lab 2
10 ml sample	0.01 mg DPD powder
1 “pump” DPD powder	~ 1 ml sample
Shake to dissolve	swirl
Within 1 minute	Check in 2 – 5 seconds
Read if color	
0.2 mg/L “free”	ND
< 0.1 Sensafe strip	

Problems associated with the DPD test

- Different handling of dissolving tablets, powder or liquids will have an impact on the result of a test.
- This is more critical with DPD than other reactions.
- DPD can be over oxidized, the second step of a DPD oxidation leads to a colorless form.
- Altering the procedure may lead to slightly different colors and different results.
- The same is true for tablets, powder and liquids. It makes a difference if you change the handling procedure (e.g. adding powder first, than adding sample, slow and fast dissolving of the powder etc....)

Labs are required to “spot check” preservation status of samples

- With no standardized procedures results will vary
- Two labs have very different procedures for “free chlorine”
- The DPD, free chlorine test, is not interference free
- Immediate means different things to different people
- How do you verify accuracy of a spot test?

For standardization between labs, should spot tests be standardized?

ASTM Work Item WK65181
Standard Practice for estimating pH to verify preservation status of laboratory samples

Chlorine?

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Questions?

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