

# Implications of Sample Preservation and Interferences in Cyanide Analysis

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## Introduction

A sample taken for chemical analysis is supposed to be a snapshot of the quantity of analyte present at the time the sample was taken. Reactions of the analyte with other constituents in the sample matrix cannot be allowed to proceed, nor can loss of the analyte by evaporation, precipitation, or oxidation. **The act of adding a chemical and refrigeration is intended to preserve the analyte concentration.**

Unfortunately, **it is possible that the very act of attempting to preserve an analyte concentration in the sample bottle actually makes matters worse.** Depending on conditions and the sample matrix itself our attempts at stabilizing what we are looking for generates more of it. Sometimes we cause it to disappear. But even worse, sometimes we are killing it and creating it all at the same time within that one little bottle.

It would not be so bad except for decisions are made on the measurements finally made. These decisions could result in fines for analyte concentrations that weren't really there, or a false assurance that analyte was absent when it really wasn't.

Other problems arise on questions of **compliance**. In the regulated community not following sampling and preservation protocol means sample collection was not valid. Changing the sample collection procedure is only allowed if the laboratory has data to support the change, however, in most cases laboratories are unable to fully characterize the matrix of a sample prior to sample collection.

We will look into the potential interferences that impact cyanide analysis and what can be done to minimize these impacts.

## Analysis of Cyanide Species

Cyanide methods are developed as an attempt to measure various cyanide species. These species range from the most toxic free cyanide to the ultra conservative estimate of cyanide toxicity we know as total cyanide. Total cyanide measurements include free cyanide, available cyanide, and non-toxic strong metal complexes. Also included in the definition of total cyanide are insoluble particulate or colloidal cyanide complexes.

The method that the samples are being collected for needs to be known at the time of sample collection. In most instances total cyanide will be analyzed.

We will be discussing protocols for sampling and preservation of cyanide defined in Part 136, however, since these potential interferences apply to all samples this discussion should be applicable to all intended uses of data and to all cyanide methods.

Table II 40 CFR Part 136.3

Parameter No./name	Container <sup>1</sup>	Preservation <sup>2,3</sup>	Maximum holding time <sup>4</sup>
23–24. Cyanide, total or available (or CATC)	P, FP, G	Cool, $\leq 6^{\circ}\text{C}$ <sup>18</sup> , NaOH to $\text{pH} > 12$ <sup>6</sup> , reducing agent <sup>5</sup>	14 days.

This is the Table II Required Containers, Preservation Techniques, and Holding Times for which most people especially Clean Water Act monitoring (NPDES), must comply. This table is an almost universally accepted protocol for cyanide sampling.

We will pay close attention to the footnotes because everything in this table and footnotes over-rides what is written in the methods. I must point out, however, that samplers rarely read the methods. Most people, in fact, follow what is on this table without reading the footnotes.

## **Containers**

The only requirements are polyethylene, fluoropolymer, or glass with no requirement for protection from exposure to UV light.

Based on current, updated holding time and interference data I suggest [amber glass containers with limited headspace](#).

## **Preservation**

Footnote 2 allow preservatives to be added to the sample bottle prior to collection provided they do not compromise the integrity of the sample, however, adding the preservative to the bottle beforehand implies that all potential interferences in the sample are known and can be treated with ease.

[Cyanide samples should not be composited since there is no way to guarantee that one grab will not react with the next.](#)

The table requires a pH greater than 12, however, footnote 3 says the pH cannot be greater than 12.3. This either requires a pH meter in the field, or the sampler's pre-knowledge of how much NaOH is required to get the pH between 12.0 and 12.3.

**Note: A 0.08% NaOH solution corresponds to 2 milliliters of a 10 M NaOH per liter sample.**

[Oxidizers must be removed](#) immediately since they rapidly react with CN decreasing its concentration. Starch iodide test strips, or field portable DPD kits determine the presence of oxidizers.

[Oxidizers must be removed prior to any pH adjustment](#) or they will rapidly oxidize any free and most available cyanide present.

Do not add a reducing agent unless oxidizers are detected, or known to be present.

## Using Ascorbic Acid is bad.

A cursory literature search on the web of laboratory SOP's reveals that ascorbic acid and pH adjustment to 12 - 13 is the most commonly practiced preservation technique for cyanide samples.

This is unfortunate because:

- a) Ascorbic acid is a carbon source that can actually **be a precursor for CN generation during storage.**
- b) Multiple holding time studies have demonstrated that **samples containing CN and ascorbic acid rapidly lose CN upon storage at high pH.**
- c) Use of **ascorbic acid combined with hydroxide both destroys cyanide and creates it.**

If ascorbic acid is used for dechlorination the holding time is **reduced to about 24 hours.** For example, a synthetic sample prepared at 200 ppb CN with ascorbic acid added and the pH adjusted to 12 recovered less than 25 % of the original cyanide present after storage for 3 days.

## Ascorbic Acid + NaOH with Cyanide present in sample

–Holding time decreased to **1 day!**

–A synthetic sample containing CN + ascorbic acid held for 3 days at pH 12 resulted in a **24 %** recovery.

## Ascorbic Acid + NaOH and no CN present

–Ascorbic acid along with a nitrogen source, or thiocyanate can react at pH 12 to create CN.

–Synthetic samples containing a carbon source, a nitrogen source, ascorbic acid, adjusted to pH 12 with NaOH **generated 5.0 – 50 ppb CN upon storage.**

### **Reducing Agents for Oxidizers**

Boiling hot sulfuric acid solution containing **thiosulfate** generates colloidal sulfur and sulfur dioxide. Sulfur dioxide distills into the absorber solution. If the absorber solution is analyzed immediately, and chloramine T is doubled 80% recovery is possible. However, as solutions sit the SO<sub>2</sub>, now sulfite reacts in the basic solution with the NaCN oxidizing it to cyanate and lowering recovery. Therefore, **if thiosulfate is suspected to be present, samples need to be analyzed as soon as possible after distillation,** and the analyst needs to verify that the amount of chloramine T added is enough to guarantee chlorine residual. This means that automated methods that use colorimetry (335.3 and Kelada 01) should not be used because there is no way the analyst can verify that enough chloramine T was added.

**Sodium arsenite has been demonstrated as an effective preservative** in most cases; however, a few studies have found slight false positives when combining sodium arsenite with distillation methods. Since sodium arsenite is an arsenic compound no one really wants to carry it around in the field, or be adding it to sample bottles.

[Sodium borohydride](#) is mentioned in the Kelada 01 method. There are legitimate concerns with its use since it [generates hydrogen gas upon acidification](#). Anyone familiar with analyzing arsenic and selenium by hydride generation is familiar with this. Since distillations are taking place near a heat source the hydrogen generation could result in an explosion hazard. Also, rapid generation of hydrogen gas within a digestion vessel could result in exploding vessels. This is especially likely with a closed vessel such as the Lachat Microdist.

Footnote 6 describes guidance/actions to take if the following potential interferences are present:

- Elemental Sulfur
- Sulfide
- Sulfite, thiosulfate, thiocyanate
- Aldehydes
- Carbonate
- Oxidizers
- Particulates

Footnote 6 is divided into 7 parts. An introduction that includes guidance for samples that can be analyzed within 48 hours, and then instructions for samples that will not be analyzed within 48 hours.

[Samples that will not be analyzed within 48 hours must be treated for the listed interferences](#). Oxidizers were covered in footnote 5, and the text on oxidizers in footnote 6 refers to footnote 5.

[Aldehydes, if present, should be treated immediately even if sample is to be analyzed within 48 hours](#). This contradicts the CFR. Also, if colloidal elemental sulfur is present, it should be removed immediately by filtration.

## Footnote 6 says

- Collect a volume sufficient for the method used.
- Adjust to pH >12 if no Sulfide
  - Analyze within 48 hours
- Otherwise treat for interferences, adjust to pH >12 and analyze within 14 days
  - Treatment must be within 15 minutes.

Most of these procedures have been embedded in methods for years. The Method Update Rule issued March 12, 2007 is the first time these procedures have been placed in the CFR.

## Elemental Sulfur

Colloidal elemental sulfur reacts rapidly with CN in basic solution to form thiocyanate. Non-colloidal elemental sulfur (that you can see) does not react rapidly. The problem is that colloidal sulfur is difficult to filter, and there are no spot tests to indicate whether or not it is there.

Caution must be used during filtration. Cyanide can be lost from low pH samples when using vacuum filtration.

## Sulfide

Samples that contain sulfide at concentrations above 50 ppm lose significant amounts of cyanide within 24 hours. Once Sulfide is reduced below 50 ppm the holding time can be extended. Even so, **samples should be analyzed as soon as possible and preferably with a method that uses on-line sulfide abatement such as ASTM D6888-04 or OIA 1678.** In fact, with slight reagent modification, ASTM D6888-04 and OIA 1678 can handle sulfide concentrations up to 200 ppm. However, remember that cyanide concentrations are rapidly depleting as cyanide remains in contact with sulfide.

The methods listed in the CFR for sulfide removal don't work. Headspace expelling and dynamic stripping leave residual sulfide behind, which interferes with distillation and analysis. The [headspace and stripping methods are difficult to use and essentially require a mobile laboratory](#). pH adjustments, as well as flow rates, must be precise. Since the methods are volatilizing high levels of sulfide these procedures must be done under a hood, or with plenty of ventilation. These procedures are removing sulfide by generating hydrogen sulfide gas.

Precipitation with cadmium in the presence of iron cyanide complexes forms a very stable and insoluble cadmium iron cyanide complex. When the cadmium sulfide is filtered off so is the iron cyanide. Mercury cyanide (a WAD, CATC, or Available cyanide species) is also lost by precipitation with cadmium.

#### Recoveries After Sulfide Removal

<b>Sample Pretreatment 200 ppm S + 200ppb CN</b>	<b>Recovery after 2 days storage at 4 °C</b>
Headspace	48%
Dynamic Stripping	55%
Cadmium Chloride	50%
Dilution	101%

As indicated by the data, [the only sulfide removal procedure that recovers CN quantitatively is dilution](#) of the sample till the lead acetate test strips no longer detect sulfide. In this case the dilution was a factor of 10. This means that the actual cyanide measured in the diluted sample was 20 ppb.

An argument against dilution is the increase in detection limit; however, most [automated](#) methods are sufficiently sensitive so that dilutions of up to 10 X still allow detection at about 5 ppb. Also, besides diluting sulfide other interferences are being diluted as well.

The only way to remove sulfide is to dilute the sample till sulfide is no longer detected on the lead acetate test strips. Then analyze the sample ASAP by a method that utilizes on-line sulfide abatement (ASTM D6888-04, OIA 1677, OIA 1678). If samples must be distilled, ASTM D7284 was developed specifically to handle samples containing sulfide. This method utilizes gas-diffusion amperometry as the measurement step after samples are distilled.

### **Sulfite, Thiosulfate, and Thiocyanate**

40 CFR Part 136 specifically states that if sulfite, thiosulfate, or thiocyanate is thought to be present to use a UV digestion method, or a non-distillation gas-diffusion method. Many people have problems with this statement because at present there are no commercial suppliers of the Kelada, and OIA 1677 is not a total method.

The intent of this statement was that since distilled colorimetrically determined cyanide results from samples that contain these substances couldn't be trusted; an available cyanide result by method 1677 is a more accurate estimate of toxic cyanide than what is possible by distillation/colorimetry.

The problem with naming the Kelada method is that it is a distillation/colorimetric method, and **does not eliminate**, or even significantly minimize, the interferences experienced because of these compounds. More so, since the Kelada is an automated colorimetric method any sulfur dioxide that distills into the absorber solution can react with cyanide forming cyanate and react with chloramine T increasing the chlorine demand. In effect, any samples that contain sulfite cannot be determined by the Kelada method. This is **evidenced by a similar method ISO 14403 that states that sulfite concentrations higher than 1 mg/l interfere.**

Remember that thiosulfate reacts under heated acid conditions (distillation) to elemental sulfur and sulfur dioxide. **Since the Kelada method is a distillation method, samples that contain thiosulfate cannot be analyzed.** In fact the Kelada method says that thiosulfate was evaluated for oxidant removal and caused an interference with the method.

Thiocyanate in the presence of nitrate or nitrite reacts to generate cyanide. Sulfamic acid has been used to minimize this effect. Thiocyanate alone reacts with the small amounts of oxidant that form due to irradiation and generate cyanides as well. **The Kelada method suggests an alkaline digest be used in the presence of thiocyanate to minimize degradation of thiocyanate to cyanide. However, if sulfite is also present in the sample, contact of sulfite and cyanide in alkaline solution rapidly oxidizes cyanide to cyanate.**

#### **Flexibility Text within Footnote 6**

*“There may be interferences that are not mitigated by approved procedures. Any procedure for removal or suppression of an interference may be employed, provided the laboratory demonstrates that it more accurately measures cyanide.”*

This statement is buried within the introductory paragraph of footnote 6 to Table II in 40 CFR Part 136. **These two sentences say that approved methods may not adequately correct for interferences and any procedure that works better may be used if the laboratory can demonstrate that it more accurately measures CN.**

The EPA does not specify, nor does it rule out, whether the other procedure that more accurately measures cyanide can be a method that is not listed in 40 CFR Part 136. The EPA, also, does not specify the procedures used to verify accuracy. **What cannot be used to verify accuracy is a comparison of the method to distillation/colorimetry because the inaccuracy of distillation based cyanide methods is well documented in literature that pre dates the promulgation of distillation by EPA.**

**Another technique commonly used to verify accuracy is spike sample recovery, however, even acceptable matrix spikes do not guarantee that results generated by a method are accurate.**

Using Matrix Spikes to Demonstrate Accuracy

Method	Technique	Recovery
335.4	Distillation/GD-amperometry	98 %
335.3	Automated distillation/colorimetry	98 %

Both methods obtained excellent, near 100% recovery indicating that results obtained were acceptable. Both methods analyzed the same sample. **The method 335.4 results were about 30 ppb and the method 335.3 (similar to Kelada method) result was about 15 ppb.**

**This synthetic sample did not contain cyanide!**

## Verify Accuracy Using Interference Free Methods

Methods demonstrated by literature and multiple users to be interference free

–OIA 1677 or ASTM D6888-04

–ASTM D 7284-08\*

–OIA 1678

[ASTM D 7284](#) analyzes total cyanide after distillation by gas-diffusion amperometry. The method has been validated by extensive single laboratory studies and has been evaluated for performance in the presence of multiple interferences. The method was developed specifically to overcome sulfide interferences with colorimetry, but in the process of evaluating interferences it was found to overcome sulfite and thiosulfate interferences as well. A modification of the currently published procedure eliminates, or at least significantly minimizes thiocyanate plus nitrate interferences.

[OIA 1678](#) is a UV irradiation gas-diffusion amperometry method. It differs from the Kelada and EPA 335.3 methods because no heat is necessary to separate CN from the acidified matrix. Earlier literature documents that automated distillation alone only liberates “free” cyanide and that in automated methods UV irradiation is needed to analyze total cyanide. [OIA 1678](#) relies on gas diffusion instead of distillation to separate cyanide from the acidified matrix. Since [OIA 1678](#) does not need heat the interferences are minimized.

**Methods OIA 1678 or ASTM D7284-08 should be used for footnote 6 sections 3 when total cyanide needs to be analyzed in the presence of sulfite, thiosulfate, or thiocyanate.**

## **Summary on Sampling, Preservation and Analysis of Cyanide**

Fill an amber 40 ml VOA vial with sample, refrigerate, and ship to the lab.

Adjust pH, add ligands, and analyze Available CN by OIA 1677 or ASTM D6888-04.

Analyze Total CN by OIA 1678 or ASTM D 7284