O·I·Analytical

Automation of Wet Chemical Analysis Methods

Application Note 21150205

Keywords
CNSolution FS 3100
DA 3500
Discrete Analysis
Ion Chromatography
FIA
Flow Injection Analysis
Flow Solution
Segmented Flow Analysis
SFA

Manual Colorimetric Analysis

Before the introduction of the visible spectrophotometer in 1933, automation in most laboratories consisted of a vacuum filtration apparatus, buret dispensers, wooden racks of test tubes, and a visual colorimeter.[1] Analysis methods emphasized volumetric and gravimetric techniques that did not require instrumentation. The advent of the visible spectrometer (Figure 1) made certain chemical determinations easier and faster, but sample preparation prior to color measurement was labor-intensive and time-consuming. Despite its drawbacks, manual colorimetric analysis is still used today and has value for laboratories performing a few determinations per day or a large variety of determinations on a few samples.

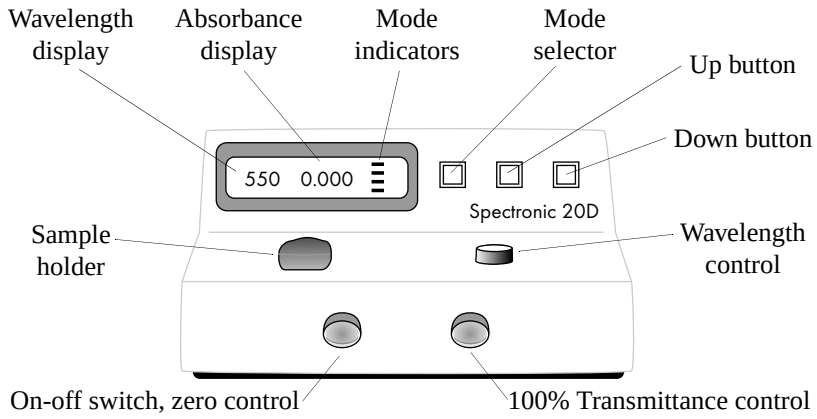

Figure 1. Spectronic 20 manual spectrophotometer

Segmented Flow Analysis

In 1957, Technicon introduced the AutoAnalyzer.[2] This instrument (Figure 2) was the first to fully automate color chemistry from reagent addition, to color development, to analytical signal recording. The AutoAnalyzer's design was modular, consisting of a turntable-type autosampler and a pump that carried reagents through plastic tubing and mixed them in glass coils. A colorimeter performed absorbance readings on flowing solutions and a strip-chart recorder reported the signal. Air segments added to the solutions in the tubing minimized dispersion of the sample during mixing and made each individual portion of sample solution resemble the reactions that occurred during color development of a manual method using beakers and test tubes. Because the chemistry was essentially the same, adapting any manual colorimetric method to the AutoAnalyzer was relatively easy.

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Figure 2. Technicon AutoAnalyzer

The OI Analytical Flow Solution IV (Figure 3) can be used for segmented flow analysis (SFA). Although similar in design to the original AutoAnalyzer, the Flow Solution IV includes more advanced capabilities such as a random access autosampler, on-line distillation, on-line UV sample digestion, automatic dilutor, and a variety of detectors including visible colorimetric, amphometric, and ion-selective electrodes.

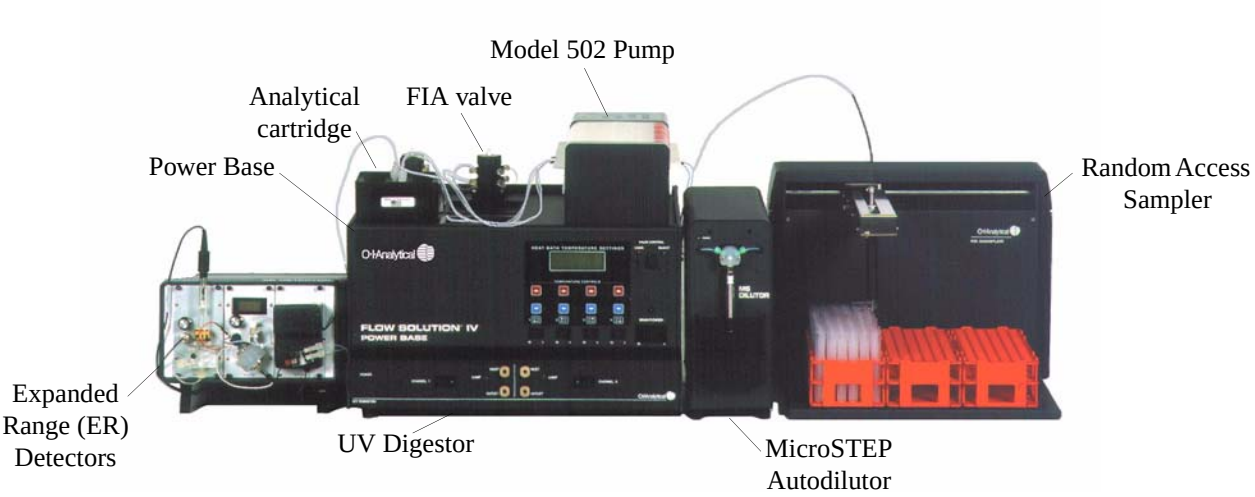

Figure 3. Flow Solution IV

SFA methods are generally preferred over manual methods because they are more precise, are independent of timed reactions, avoid tedious repetitive steps, and are relatively faster. They also consume less sample, and in multichannel systems, several parameters can be analyzed simultaneously from one sample cup. Analysis quality improves by eliminating human errors that can occur during manual tests.

The basic principle of SFA consists of a continuous flowing stream of sample segments that are separated by air bubbles introduced into the tubing. The air bubbles allow complete mixing of the sample as it travels through tubing, mixing coils, and any reactors. Air bubbles also “scrub” the sides of the tubing reducing carryover of sample into the following sample segments (Figure 4).

Figure 4. A section of tubing showing air bubbles and sample mixing

Because the presence of bubbles decreases carryover, long reaction times are possible with SFA allowing analytes with slow reaction kinetics to go to completion (steady state) prior to sample measurement. Reaching steady state ensures the analysis is at equilibrium and is at the maximum attainable signal for a set of conditions (Figure 5 and Figure 6). Reading results at steady state allows lower detection limits and better precision.

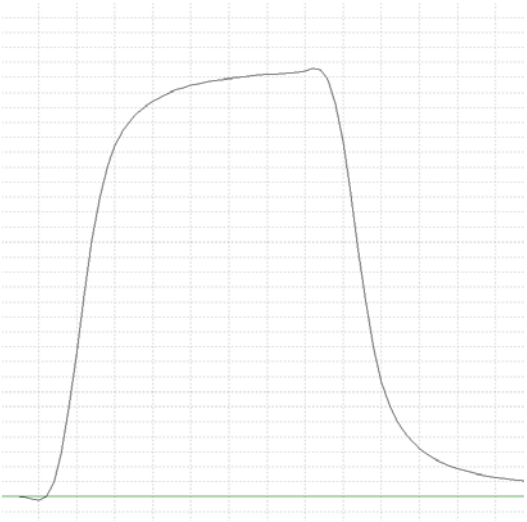

Figure 5. A peak at steady state on the Flow Solution IV

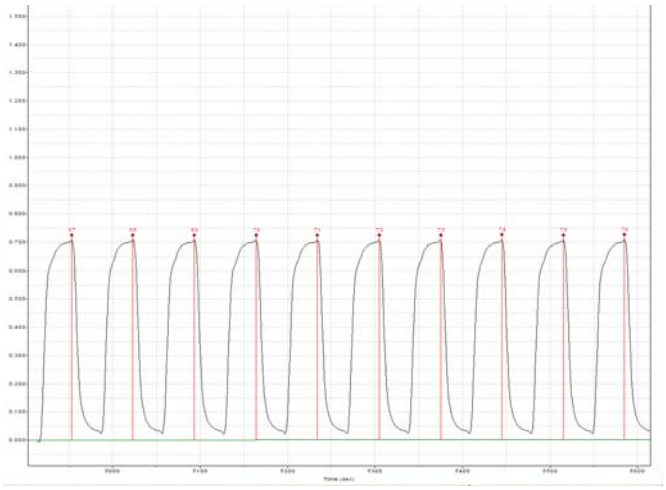

Figure 6. A series of steady state peaks on the Flow Solution IV. Note the reagent steady state peaks is an upside down mirror image of the sample steady state.[3]

Flow Injection Analysis

In 1975, Ruzicka and Hansen discovered that using air to avoid dispersion became unnecessary by decreasing the tubing diameter.[3] Sample solutions could be introduced using a valve and pulled into the reagent stream instead of being pushed in by the pump. This method had advantages such as less reagent use (although more sample consumption) but more importantly an accurately-reproducible sample volume. This method was termed flow injection analysis (FIA).[4] A typical flow injection instrument consists of an autosampler, pump, injection valve, mixing coils, heaters, and detector. The CNSolution™ FS 3100 (Figure 7), Flow Solution 3000, and Flow Solution IV can run FIA methods.

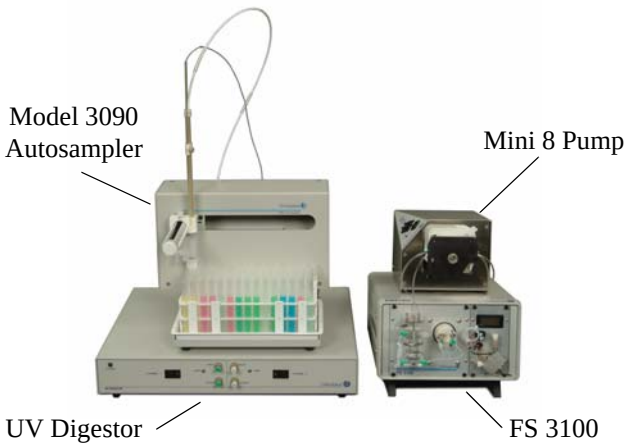

Figure 7. CNSolution FS 3100

The underlying principle of FIA is continuous forward flow, also called laminar flow. As the sample plug flows down the tubing, the friction at the tubing walls causes the solution at the center of the tube to travel faster than the solution touching the walls of the tubing. This is called axial dispersion (Figure 8).

Figure 8. Axial dispersion

To allow sample and reagent to mix and react properly, mixing loops or obstructions are placed in the flow path, causing radial dispersion (Figure 9). Because no air bubbles are present to prevent carryover, tubing must be kept as short as possible. This results in fast sample analysis times, but limits FIA to analytes with fast reaction kinetics.

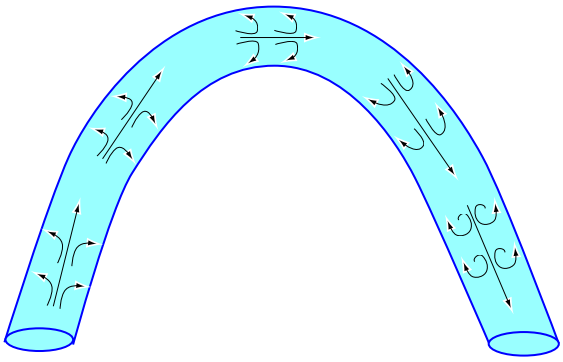

Figure 9. Radial dispersion

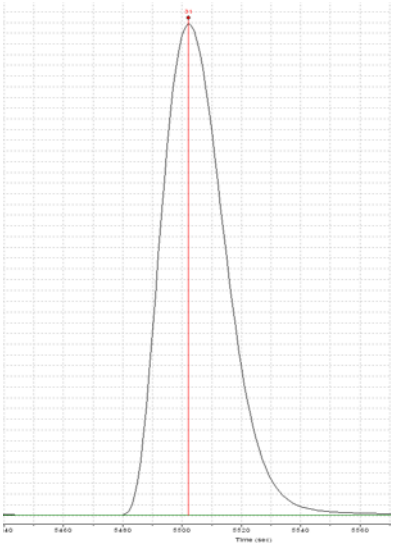

Figure 10. FIA peak. Note that the signal does not reach steady state.

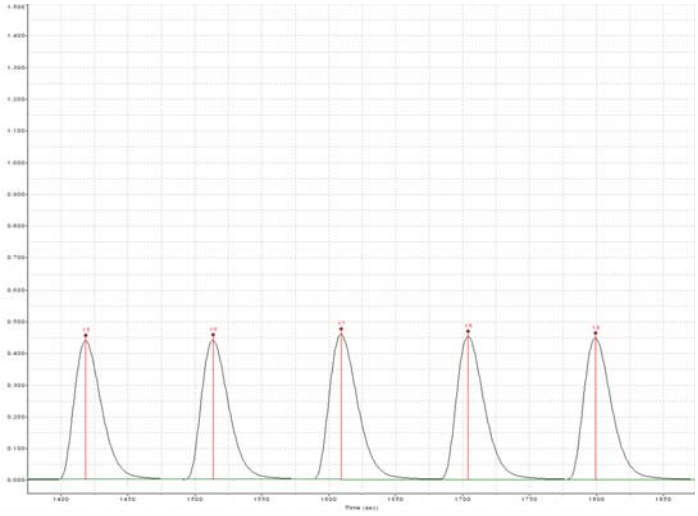

Figure 11. A series of FIA peaks, which are not mirror images.

Comparing SFA and FIA

Table 1. Advantages and disadvantages of SFA and FIA

FIA Advantages	SFA Advantages	FIA Disadvantages	SFA Disadvantages
• Faster analysis times, usually 30–60 seconds • Simple and reliable, best with only one or two reagent additions • No need for surfactant and bubbles • Easier to change from one method to another	• Very stable • Slow reactions can go to completion 2–5 minutes and longer reaction times (on-line distillations and digestions, on-line neutralizations like TKN and total phosphorous) • Less subject to interference in samples that depend on reaction rate • Easy to troubleshoot poor flow patterns, can see bubbles	• Only possible with fast reaction times of <two minutes (nitrite, phosphate, chloride) • Does not reach steady state (60–90% of steady state signal) • Difficult to troubleshoot poor flow patterns, cannot see bubbles	• Sample throughput usually 30–60 samples per hour • Some chemistries require debubbling and rebubbling

Discrete Analyzers

At about the same time the Technicon AutoAnalyzer was introduced, a robotic chemistry analyzer that performed colorimetric methods in discrete sample cups was developed.[5] This analyzer never became popular, probably due to limitations in computerization, but it is the ancestor of the modern discrete automatic chemistry analyzer.

The OI Analytical DA 3500 Discrete Analyzer (Figure 12) is a compact instrument relative to segmented and flow injection systems. It automatically adds sample aliquots and reagent to a small cell, mixes, waits for reaction to occur, and then measures the analyte. The discrete analyzer uses only microliter amounts of sample and reagents and consumes only the amount of reagent required for each test, generating less waste and requiring less sample to be shipped to the laboratory. The discrete analyzer has true “walk-away” capability with no flows, baselines, peak shapes, or pump tubing that require monitoring. It uses the same reagents, sample-to-reagent ratios, and analytical wavelengths as manual methods.

Figure 12. DA 3500 Discrete Analyzer

Presently, discrete analyzers can only run simple chemistries and time-consuming sample preparation steps such as distillations, digestions, and matrix removal or enhancement must still be done manually. Flow injection analyzers perform the same chemistries as discrete analyzers, but discrete analyzers do not perform the same chemistries as flow injection analyzers. The discrete analyzer cannot run gas diffusion, dialysis, or on-line distillations and digestions. It cannot perform complex chemistries. For complex chemistries, the segmented flow analyzer still has the advantage.

Discrete analyzers require very little staff training. Manufacturers sell products with methods preinstalled and reagents already prepared. Analysts only have to select a method, label sample sites, and press start.

Many of the colorimetric manual chemistries for approved methods have been removed from the USEPA-approved methods for the National Pollutant Discharge Elimination System (NPDES). The manual colorimetric method for ammonia that is similar to the flow method, for instance, is not approved. A manual Nessler reagent method for ammonia is approved but requires manual distillation.

Ion Chromatography

Ion chromatography is similar to flow injection analysis in some ways. The sample is injected using a valve into a flowing stream of reagent within small diameter tubing. Ion chromatography separates analytes and measures them separately, while FIA mixes the sample and reagents together and measures a reaction product. Ion chromatography can separate ions, react them chemically, and determine concentrations of individual reaction products from a single sample injection. Thus, with ion chromatography, multiple analytes can be determined from one sample injection.

One of the most common uses of ion chromatography is determining anions in aqueous samples (Figure 13). With one injection, the most common anions in water can be accurately measured in 10–15 minutes. Anion chromatography is used extensively in analyzing anions and disinfection by-products in drinking water.

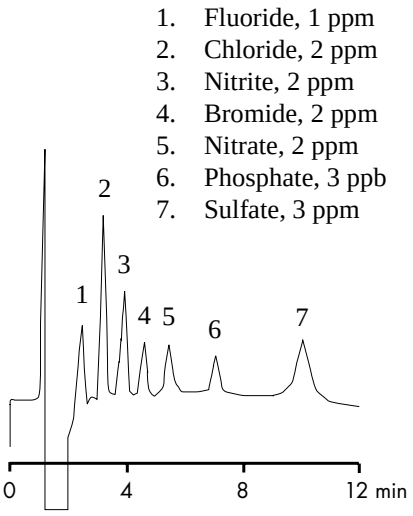

Figure 13. Ion chromatogram

The typical ion chromatograph consists of an autosampler, pump, injection valve, column and guard column, suppressor, and detector. Analyte separation takes place on the column, while the guard column helps to prevent column contamination. The suppressor converts the analyte to a more readily-detected form.

Ion chromatography is an excellent technique for determining anions in drinking water, and relatively clean groundwater and wastewater. In samples of unknown matrices, conductivity should first be determined so dilutions can be made prior to analysis. These dilutions are necessary to avoid overlap of large peaks with smaller peaks and to bring large amounts of analyte (usually chloride or sulfate) within calibration range. Unfortunately, this necessary predilution sometimes causes other analytes to be diluted below detectable levels. A chemical method such as SFA, FIA, or discrete analysis rarely requires predilution to remove interferences.

In ion chromatography large chloride peaks can overlap nitrite peaks making determination difficult. Chloride does not interfere in chemical methods.

Large sulfate peaks can cause retention time shifts in ion chromatography and actually be measured as phosphate. The sample must be diluted and reanalyzed for sulfate, but this causes loss of phosphate numbers. Sulfate does not interfere with phosphate in chemical methods.

Acidic samples and samples containing high concentrations of trace metals cannot be analyzed without significant dilution. Acidic samples, besides having large amounts of the acid anion, cause retention time shifts resulting in misidentification of analyte peaks. Trace metals occupy active sites on the column, eventually ruining its ability to adequately separate ions. Chemical methods are typically developed to avoid interferences from acidity and trace metals. For instance, low chloride levels can be determined chemically in the presence of very high concentrations of iron, aluminum, copper, and sulfuric acid.

Although ion chromatography performs well analyzing nitrite in drinking water, the short holding time requires setting up chromatographic runs specifically for the analysis of this ion before its holding time expires. If off-scale chloride or sulfate signals occur, the sample must be rerun. Chemical methods are more suited to quickly analyze short holding time parameters such as nitrite and phosphate. Samples preserved for nitrate can be analyzed by

chemical methods but not by ion chromatography.

Table 2. Comparison of various wet chemical analysis methods

Parameter	Flow Solution 3000	Flow Solution IV	DA 3500 Discrete Analyzer	Ion Chromatography
Bromide	✓	✓	✓	✓*
Chloride	✓	✓	✓	✓*
Fluoride	✓	✓	✓	✓*
Phosphate	✓	✓	✓	✓*
Nitrate	✓	✓	✓	✓*
Nitrite	✓	✓	✓	✓*
Sulfate	✓	✓	✓	✓*
Ammonia	✓	✓	✓	✓**
Chromium(VI)	✓	✓	✓	✓**
Post-distillation cyanide	✓	✓	✓	✓**
Post-digestion total phosphorous	✓	✓	✓	
Post-distillation TKN	✓	✓	✓	✓**
Post-distillation phenol		✓	✓	
Silica	✓	✓	✓	
Hardness	✓	✓	✓	✓**
Color	✓	✓	✓	
Sulfide	✓	✓	✓	
Sulfite	✓	✓	✓	
Total cyanide	✓	✓		
Weak acid dissociable (WAD) cyanide	✓	✓		
On-line distillation cyanide		✓		
On-line distillation phenol		✓		
On-line digestion nitrogen		✓		
On-line digestion phosphorous		✓		
TKN by gas diffusion	✓	✓		
Ammonia by gas diffusion	✓	✓		

*Uses anion chromatography.
** Uses cation chromatography, requiring different column and conditions than anion chromatography.

Limitations of Automated Wet Chemical Methods

- The analyzer cannot weigh solid samples. It cannot extract samples.
- The analyzer cannot guarantee the purity of calibration standards.
- The analzer cannot determine the accuracy of a method for each matrix.

Grouping of Test Parameters by Matrix and Holding Time for Maximum Efficiency

If the laboratory uses automated methods to save labor costs and decrease turnaround times, the laboratory should attempt to analyze as many analytes from one sample cup as possible. As discussed previously, ion chromatography can determine multiple analytes from one sample injection. Unfortunately, interferences present and a wide range of analyte concentrations typically cause the analyst using ion chromatography to repeat tests with diluted samples to bring some of the analytes within scale. Also, when eliminating interferences by predilution, at times the ion chromatography data can be useless for analytes that were diluted below detection limits. Using SFA, FIA, and discrete analyzers, analyzing multiple parameters from a single sample cup is possible.

The limiting factor on simultaneous determinations by SFA is the number of channels available on the instrument. FIA is limited by the number of channels, the number of injection valves, and the amount of sample in the cup. The discrete analyzer is limited by the number of available sample cuvettes and by the sample volume in the cup. Other limiting factors for water testing laboratories include holding time and sample preservation methods (sample preservation requires that certain compounds can only be analyzed out of the properly-preserved containers).

Assuming the laboratory can only determine three separate parameters at one injection due to hardware limitations, the best approach for the most rapid results is to analyze parameters with compatible matrices and the shortest holding times first. Short holding time parameters that do not require sample preservation chemicals include the following:

- Chromium(VI)
- Sulfite
- Color
- Anionic surfactants
- Sulfide
- Nitrite nitrogen
- Orthophosphate

If the laboratory performs any grouping of the above tests on multiple samples, the laboratory can set up automated systems to simultaneously (or sequentially for discrete) determine them from one sample cup. All of the above, except anionic surfactants, are fast chemistries and can be adequately determined by SFA, FIA, or discrete analyzers. Determining anionic surfactants requires solvent extraction and must be performed by flow methods.

The following parameters require analysis from a sulfuric acid-preserved sample:

- Ammonia nitrogen
- Total phosphorous
- TKN and total organic nitrogen
- Nitrate plus nitrite nitrogen
- Chemical oxygen demand
- Total phenols

Simultaneously determining nitrate plus nitrite nitrogen, and ammonia allows the laboratory to rapidly collect data on simple nutrients.

Total phosphorous and TKN require separate sample digestions, making simultaneous determinations impossible unless the laboratory is allowed to use on-line UV/persulfate digestion methods. The on-line methods are currently proposed but not approved.

Phenol requires distillation prior to analysis. On-line distillation is acceptable, but would be difficult as a simultaneous determination because of long sample processing times.

Discrete analyzers can run these methods, but with the exception of nitrate plus nitrite nitrogen and possibly ammonia, all require some sort of preliminary sample preparation.

NOTE: All colorimetric manual methods for ammonia and TKN are either not approved or require preliminary distillation. Once distilled samples can be analyzed by the same method at the same time.

Method Detection Limits and Maximum Contaminant Levels

With the exception of regulated drinking water parameters set under the Safe Drinking Water Act (SDWA) and the Contract Laboratory Program (CLP), the USEPA does not require detection limits other than those specified in the methods. Also, the USEPA-required detection limits are typically much higher than the detection limits reported in OI Analytical's and other manufacturers' literature. Table 3 lists some USEPA-required detection limits for drinking water.

Table 3. Comparison of MDLs

Method	MDL (mg/L)
Cyanide by automated distillation	0.005
Cyanide by manual distillation	0.02
Cyanide by OIA-1677	0.0005
Nitrate by automated cadmium reduction	0.05
Nitrate by ion chromatography	0.01
Nitrite by manual spectrophotometry	0.01
Nitrite by automated cadmium reduction	0.05
Nitrite by ion chromatography	0.004

Instead of setting Method Detection Limits (MDL) for each parameter, the USEPA typically sets a parameter's Maximum Contaminant Level (MCL) often on a case-by-case (or permit-by-permit) basis. Because of error involved in measurements near the MDL, laboratories usually strive to obtain an MDL at least ten times lower than the MCL. Often the laboratory reports a value higher than the MDL as a minimum-reporting limit to protect itself and its client from false positives. Table 4 lists some MCLs for drinking water.

Table 4. Comparison of MCLs

Parameter	MCL (mg/L)
Sulfide	0.05
Chloride	250
Fluoride	2
Nitrate nitrogen	10
Nitrite nitrogen	1.0
Sulfate	250
Surfactants	0.5
Cyanide	0.2

Each state may also set or require minimum detection limits for data submitted to that state. These limits can be more strict or equal to limits set by USEPA. Usually, however, a state cannot require laboratories to report data below the minimum applicable range specified by USEPA in the USEPA-approved method.

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