Automated nitrate analysis of water

Application Note

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Introduction

Analytical laboratories may analyze thousands of samples a year using UV-Vis spectrophotometers. Before these samples can be analyzed, standards must be prepared and the samples may require one or more reagents to be added. During the analysis of a batch of samples there may be over range samples. If so, the samples must be diluted and re-analyzed.

These tasks can now be completely automated by using the Sample Preparation System (SPS-5).

In this study the following topics are discussed:

- Automated standard and sample preparation versus manual standard and sample preparation
- Accuracy of automated sample analysis versus manual sample analysis
- Accuracy of over range dilutions
- Precision of the SPS-5
- Time required for automated and manual analysis

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Experimental

Equipment

- Cary 1E UV-Vis spectrophotometer
- Routine Sampler Accessory with a 10 mm flow through cell
- SPS-5 with diluter
- Concentration Application software
- Autodilutor Application software
- Type 10, 25, 50 or A type sample racks
- 10, 25, 50 or 150 mL size test tubes

Reagents

- Potassium nitrate (Analytical Reagent)
- 37% m/v Hydrochloric acid (Analytical Reagent)
- Chloroform (Analytical Reagent)
- Water - distilled and de-ionized

Method

For this study, the American Public Health Association (APHA) Nitrate method[1] was used. This method measures the NO_3 content in water samples and is recommended for screening samples that have low organic matter content. The absorbance of the sample is measured at two wavelengths and a simple calculation is performed.

The absorbance is measured at 220 nm and a second reading is taken at 275 nm. This allows correction for the interference due to dissolved organic matter. The difference between the two absorbance measurements is then calculated by the formula:

$$Abs_{220} - 2*(Abs_{275})$$

This is easily performed with the Cary by entering the following ADL command on the *User_Result* line on the Advanced Parameters page:

READ(220)-2*READ(275)

Preparation of stock nitrate solution (10 µg N/mL)

KNO_3 was oven dried at 105 °C for 24 hours and kept in a desiccator. The salt (0.7218 g) was dissolved in approximately 500 mL water. CH_3Cl (2 mL) was added as a preservative. The flask was put in an ultrasonic bath to speed the dissolution process. The solution was diluted to 1000 mL with water.

Preparation of standard nitrate solution (10 µg N/mL)

1. **Manual preparation:** 20.00 mL of the stock nitrate solution was diluted to 200 mL with water.
2. **Automated preparation:** 4.000 mL of the stock nitrate solution was diluted to 40.00 mL with water using the Autodiluter Application Software Samples page.

The stock nitrate standard was placed in the source rack. The destination rack used was a type 25, 50 or 100 mL depending on the volume of standard solution required.

Preparation of 1 N Hydrochloric acid

1. **Manual preparation**: 8.0 mL of HCl was diluted to 100 mL with water.
2. **Automated preparation**: 8.291 mL of HCl was diluted to 100 mL with water using the Autodiluter Application Software Samples page. See Figure 1 for the values used. Care needs to be taken when using concentrated HCl. The SPS-5 was fitted with the front cover and the rear cover/fume hood to minimize dispersal of the acid fumes.

Calibration standards

1. **Manual preparation**:

Table 1. The above dilution scheme was used to prepare the calibration standards

Standard NO_3 (mL)	1 N HCl (mL)	Final Volume (mL)	NO_3 Concentration (µg/mL)
1	2	100	0.10
2	2	100	0.20
6	2	100	0.60
10	2	100	1.00
15	2	100	1.50
30	1	50	6.00

2. Automated preparation: Figure 2 shows the parameters used for the Standard page of the Autodiluter Application to prepare the calibration standards.

The standard nitrate was placed in the source rack. Component 1 was the 1 N HCl.

Sample preparation

1 mL of 1 N HCl was added to 50 mL of sample. The samples used for this evaluation were prepared standards of known values. The report shows the appropriate volume correction (see Figure 3). The volume correction is activated on the Advanced Parameters page of the Cary E software. The factors are entered on the Labels and Corrections page of the Cary 1E software.

If samples require filtering an acid washed, ashless, hard finished filter paper, sufficient enough to remove fine precipitates, is recommended. A Whatman Nº 542 filter paper is adequate for this task.

Instrument parameters

Some operational aspects of the SPS-5 are shown in Figures 1–5.

Figure 1 relates to the automatic preparation of 40 mL of 10 µg/mL standard solutions.

The first line on this page directs the preparation of 40 mL of 10 µg/mL standard solution. The following two lines direct the preparation of 5 and 20 µg/mL standard solutions respectively for the overrange dilution study. The fourth line prepares 1 N HCl. The fifth line was used to add 0.196 mL of 1 N HCl to 9.804 mL of sample (ensuring acid to sample ratio of 1:50) for 100 samples.

A total of 10 different dilutions or reagent additions can be performed using this page.

The program shown in Figure 2 directs the preparation of 20 mL volumes of a blank and six standard solutions[1].

Figure 3 is an example of a nitrate analysis report showing overrange samples and volume correction.

Results

Reproducibility of standard preparation

The results of the SPS-5 prepared standard solution measurements are shown in Figure 4. Absorbances and correlation data obtained for an auto run are also shown.

Figure 5 shows the results and calibration data for the manually prepared standards, together with correlation data from the manual run.

As can be seen from Figures 4 and 5, both calibration graphs have a correlation coefficient (r^2) of 1.000. This shows the accuracy of the SPS-5 standard preparation. The standards prepared with the SPS-5 are in general accordance with those recommended by ASTM. The manually prepared concentrations were restricted to the size of the pipettes available.

The standards were prepared six times by the SPS-5. They were run as samples and the results are shown in Table 2.

Table 2. Accuracy of standards prepared using the SPS-5

Expected Nitrate Conc (µg/mL)	Mean Conc Found (µg/mL)	Std Deviation	%RSD
0.100	0.101	0.006	6.0
0.200	0.198	0.005	5.1
0.600	0.598	0.003	5.0
1.000	1.001	0.006	0.6
1.400	1.398	0.007	0.5

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Press F1 for HELP, F2 to EDIT

SAMPLES PAGE

Source rack	Source tube	Dest. rack	Dest. tube	Number	Sample mL	Comp 1 mL	Comp 2 mL	Diluent mL	Final mL	Pause	Mix secs
2	1	3	1	1	4.000	0.000	0.000	36.000	40.000	NO	0
2	1	3	2	1	2.000	0.000	0.000	38.000	40.000	NO	0
2	1	3	3	1	8.000	0.000	0.000	32.000	40.000	NO	0
3	10	2	2	1	8.291	0.000	0.000	91.709	100.000	NO	0
1	1	4	1	100	9.804	0.000	0.000	0.196	10.000	NO	0
1	1	1	1	0	0.000	0.000	0.000	10.000	10.000	NO	0
1	1	1	1	0	0.000	0.000	0.000	10.000	10.000	NO	0
1	1	1	1	0	0.000	0.000	0.000	10.000	10.000	NO	0
1	1	1	1	0	0.000	0.000	0.000	10.000	10.000	NO	0
1	1	1	1	0	0.000	0.000	0.000	10.000	10.000	NO	0

Figure 1. The Autodiluter Application Samples page

CARY AUTODILUTOR APPLICATION 1.00

Press F1 for HELP, ESC to continue

STANDARDS PAGE

Standard number	Standard mL	Comp 1 mL	Comp 2 mL	Diluent mL	Final Vol mL	Final Conc ug/mL	Mix secs
Blank		0.400	0.000	19.600	20.000		0
1	0.200	0.400	0.000	19.400	20.000	0.100	0
2	0.400	0.400	0.000	19.200	20.000	0.200	0
3	1.200	0.400	0.000	18.400	20.000	0.600	0
4	2.000	0.400	0.000	17.600	20.000	1.000	0
5	2.800	0.400	0.000	16.800	20.000	1.400	0
6	14.000	0.400	0.000	5.600	20.000	7.000	0
7							
8							
9							
10							

Conc: 10.000 ug/mL Blank: YES Number of Standards: 6

Valid input range is 0.000 to 18.000

Figure 2. The Autodiluter Application Standards page

```
Method Volume 50.0000 mL
Conc Units  Unit

Rack 1   Tube 8
APHA NO3 Sample 1
Sample   Mean     Volume   Dilution Conc
-------------------------------------------
 1        1.5749  51.0000           6.9938

Rack 1   Tube 9
APHA NO3 Sample 2
Sample   Mean     Volume   Dilution Conc
-------------------------------------------
 2        2.6001  51.0000          12.2255
```

Figure 3. The Reports Display page

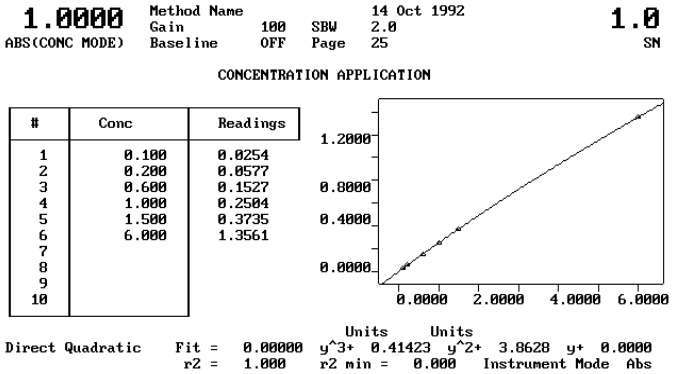

Figure 4. The Concentration Application page for SPS-5 prepared standards

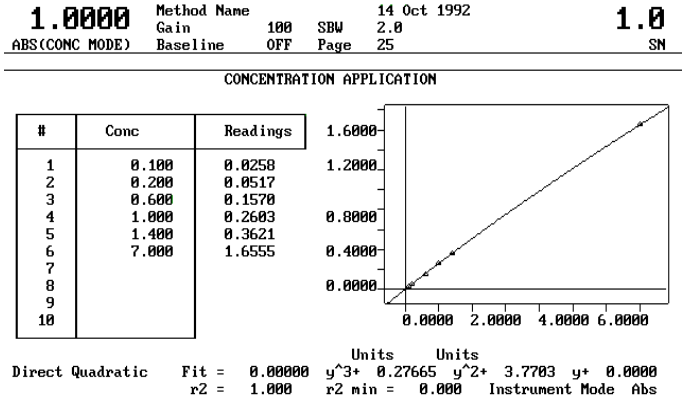

Figure 5. The Concentration Application page for manually prepared standards

The results show good reproducibility for the automated preparation and automated analysis of standards.

Overrange dilutions

Any sample with a concentration >10 % of the *Ordinate Max* will be diluted. Overrange dilutions will be performed if an overrange solution is in racks 2–5.

A 5 µg/mL nitrate standard was prepared by the SPS-5 and used for calibration. Ten nitrate solutions each of 10 and 25 µg/mL were prepared using the SPS-5 and analyzed as samples. These overrange samples were automatically diluted and the concentrations calculated. The results are shown in Table 3.

Table 3. Overrange dilution accuracy

Expected Conc (µg/mL)	Mean Conc Found (µg/mL)	Std D'vn(n=10)	%RSD	Dilution Error
10.00	10.14	0.05	0.5	1.4
25.00	25.31	0.03	0.1	1.2

One dilution was performed for the 10 µg/mL standard and two dilutions were performed for the 25 µg/mL standard. The mean diluted concentration found reflects the accuracy of the diluter. The 1.4% dilution error for analyzing the standards 10 times shows the excellent accuracy of the SPS-5. The excellent repeatability of the SPS dilution process is also apparent.

Preparation time using the SPS-5

Approximately 2.5 minutes was required to prepare 40 mL of nitrate standard. To prepare six calibration standards, including blank, required 14.5 minutes resulting in a total time of 17 minutes for calibration standard preparation.

There are distinct advantages in automated preparation. These include the following:

1. Once racks, tubes and reagents are loaded, the preparation is fully automatic, allowing the operator to perform other duties.
2. Operator errors in sample preparation are eliminated. Any variations due to different operators preparing standards is also eliminated The SPS-5 consistently prepares accurate standards.
3. The user is not restricted to availability of glass pipettes when using the SPS-5 to prepare standards and an infinite range of concentrations can be prepared.
4. Standards and samples are analyzed in the same vessel in which they were prepared. Any potential cross contamination is therefore eliminated.
5. Cleaning of glass pipettes is eliminated.
6. The time required for the automated standard preparation is constant. The time required for the manual standard preparation will vary and is operator dependent.

The excellent accuracy and precision obtained demonstrates that the built-in mixing routine has excellent agitation for standard and sample preparation.

Analysis time

When using the SPS-5, analyzing a blank, 6 standards and 7 samples requires 14½ minutes.

Once a calibration is performed and stored, recalibration is not usually required.

Using a previously stored calibration, the SPS-5 can analyze 6 samples and a blank in less than 9 minutes.

The analysis time required is greater using the SPS-5 compared to manual analysis but as the operation is unattended, the operator is free to perform other tasks. Productivity can be improved because the sampler may operate unattended outside of normal laboratory working hours.

Conclusion

Standards can be accurately prepared and samples can have reagents automatically added and thoroughly mixed. Up to 500 samples can be automatically analyzed. Any overrange solution which may be present in a batch can be automatically diluted during an analysis using the SPS-5. This will occur accurately and the uncorrected and corrected results are printed.

The SPS-5 with Autodiluter Application software is a powerful stand alone accessory which accurately and precisely prepares standards and samples. When used in conjunction with the Cary it allows analyses of samples and accurate dilutions of overrange samples to be automatically performed.

Analyses with the SPS-5 and Cary can be performed almost continuously throughout the day and night increasing sample throughput, the productivity and profitability of the laboratory.

References

1. 'Ultraviolet Spectrophotometric Screening Method'. Standard methods for the examination of water and wastewater, 16th Edition. *American Public Health Association*. Washington, **1985**, pp 392-393.

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