APPLICATION NOTE



UV/Visible Spectroscopy

Water Analysis using LAMBDA: Total Phosphorus (T-P), Ascorbic Acid Method

Introduction

In this application note, the quantitative analysis of total phosphorus (T-P)

was performed by the ascorbic acid method. Data are rapidly acquired using the LAMBDA[™] 465 UV-Vis Spectrophotometer and processed using the UV Lab[™] Software.

Principle

Total phosphoric compounds in water sample are changed to the phosphate (PO_4^{3-}) form by oxidation. After treatment with molybdate - ascorbic acid solution, blue color is created. This color is measured at 880 nm.



Reagents and Apparatus

- 1. Phosphate ion standard solution (0.005 mg P/mL)
- 2. Unknown sample
- 3. Ammonium molybdate solution
 - Dissolve 6 g ammonium molybdate tetrahydrate ((NH₄)₆Mo₇O₂₄·4H₂O) and 0.24 g antimonyl potassium tartrate (C₄H₄O₆K·SbO·1/2H₂O) in 300 mL D.I water, add 120 mL sulfuric acid (2 + 1) and 5 g ammonium sulfamate (NH₄OSO₂ NH₂), dilute to be 500 mL with D.I water.
- 4. 7.2% L-Ascorbic acid solution - Dissolve 7.2 g L-ascorbic acid ($C_6H_8O_6$) in 100 mL D.I water.
- Ammonium molybdate Ascorbic acid solution
 500 mL ammonium molybdate solution mix with 100 mL
 7.2% L-ascorbic acid solution.
- 6. LAMBDA 465 (PDA UV/Vis Spectrophotometer)
- 7. UV Lab Software
- 8. Cuvettes (10 mm pathlength)

Procedure

- Prepare serial volumes (1~20 mL) of phosphate ion standard solution (0.005 mg P/mL) in 100 mL volumetric flasks for standards, dilute to volume with D.I water. Fill 25 mL nessler tubes with each, then perform the experiment as following procedure.
- 2. Fill a 25 mL nessler tube with an unknown sample.
- 3. Add 2 mL ammonium molybdate ascorbic acid solution.
- 4. Mix and leave for 15 min at 20~40 °C.
- 5. In Quantification Standard mode, measure the absorbance of standards with reference to standard 1 (0 ppm) at 880 nm.
- 6. In Quantification Sample mode, measure the absorbance of the unknown sample and calculate its concentration.

Instrument Parameters

The instrument parameters of the LAMBDA 465 are as follows: Figure 1 shows experimental setup.

Experiment Setup

Data type:	Absorbance
Sampling:	Single cell
Mode:	(Spectra no.: 1/Scan no.: 30/Integration no.:
	1/Gain no.: 1)

Experiment Method

Use wavelength: 880 nm Curve dimension: 1

Method - C:\UV Lab\Total Phosphorus.mtd					
Experiment Type: Qua	antification Standa	rd 🖻 Open	Save		
Experiment Setup					
Data Type	Absorb	ance			
Sampling	Single	Cell Holder			
Mode	Fast				
	30				
	1				
Baseline Correction					
Quantification Standard					
Analysis Name	T_P				
Concentration Unit	ppm				
Use Wavelength (nm)	880				
Standard Replicate No.	1				
Sample Replicate No.	1				
Curve Zero Offset	Yes				
Curve Order	1				
Derivative Order	0				
J Standard Concentratio	n				
	Save as Default	<u>O</u> K	Cancel		

Figure 1. Experimental setup for T-P analysis.

Result

1. Calibration curve

Figure 2 shows spectra of the T-P standards. Table 1 and Figure 3 show the data and calibration curve of the six standards. The correlation coefficient R^2 is 0.9962.

Table 1. Calibration data of T-P standards.

No.	Name	Concentration (ppm)	AU (880.00 nm)
1	Standard 1	0.00	0.0005
2	Standard 2	0.10	0.0671
3	Standard 3	0.20	0.1232
4	Standard 4	0.50	0.3102
5	Standard 5	1.00	0.6068
6	Standard 6	2.00	01.0829

 $R^2 = 0.99620$

Function : Y = 0.5432X + 0.0211

2. Unknown sample

The concentration of an unknown sample was determined using the calibration curve shown in Figure 3. The concentration of this unknown sample is 0.53 ppm (see Table 2).

Table 2. Concentration of unknown sample.

Name	Concentration	Dilution	AU
	(ppm)	Factor	(880.00 nm)
Sample 1	0.53	1.0	0.3085



Figure 2. The spectra of T-P standards by ascorbic acid method.



Figure 3. The calibration curve of T-P standards.

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Conclusion

Using the LAMBDA 465 and UV Lab Software, quantitative analysis of total phosphorus (T-P) in water was performed. Rapid acquirement of spectra and good sensitivity were obtained with the LAMBDA 465. A good calibration curve with an R² value of 0.9962 was acquired. UV Lab Software was used effectively for quantitative analysis and to process the data efficiently.