Analysis of Oligonucleotides using the CTech™ SoloVPE® PLUS System

Application Note

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Abstract

The CTech™ SoloVPE® PLUS System is a new instrument for at-line concentration measurement. The system utilizes variable pathlength technology and the Beer-Lambert law to enable the Slope Spectroscopy® method, a powerful tool for measuring concentrations of bioprocessing samples. It has a higher dynamic range than the original CTech™ SoloVPE® System due to the finer pathlength resolution of the motor.

This study discusses the use of the CTech SoloVPE PLUS System with oligonucleotide samples to demonstrate its capability and limits of detection. The experiment tested two different oligonucleotides across three days. Each sample was tested at different dilutions, spanning a concentration range from 0.1 mg/ml to 30 mg/ml. The samples were also measured on the original SoloVPE System for comparison.

Background

The CTech SoloVPE PLUS System is an at-line analytical instrument for measuring biological product concentrations via UV-Vis absorbance spectroscopy. The operating principle is based on the Beer-Lambert law:

$$A = \varepsilon c l$$

Where A is the absorbance through the sample, ε is the extinction coefficient, c is the concentration, and l is the pathlength. Unlike traditional UV-Vis spectroscopy, which uses a fixed pathlength to measure absorbance, the SoloVPE PLUS System uses variable pathlength technology (VPT) and takes multiple absorbance measurements at several different pathlengths. Variable pathlength spectroscopy, or the Slope Spectroscopy method, uses a variation of the Beer-Lambert law:

$$m = \varepsilon c$$

Where m is the change in absorbance per unit pathlength, also called the slope. Slope-based measurements enable higher concentration readings without the need for dilution, as the SoloVPE PLUS instrument adjusts the pathlength to keep the absorbance within the detector's linear measurement range.

Materials and Methods

This study was performed in collaboration with Ionis Pharmaceuticals. All samples were provided and tested by Ionis.

- SoloVPE PLUS System (SYS-VPE-SOLO-P)
- SoloVPE System (SYS-VPE-SOLO5)
- Fibrette® Optical Components (OF0002-P50)
- SoloVPE vessels (OC0009-1) and vessel holders (HM0178)
- Oligonucleotide samples:
    - ◊ Oligo A, 30 mg/ml (neat)
    - ◊ Oligo A, 5 mg/ml (neat)
    - ◊ Oligo A, diluted to 0.1 mg/ml
    - ◊ Oligo B, diluted to 0.1 mg/ml
- CTech™ ConfiRM® Certified Slope Reference Materials (MRM-80-B5)

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The oligonucleotide samples were measured using a wavelength of 260 nm. Oligo A were measured at neat concentrations of 30 mg/ml and 5 mg/ml, then diluted to 0.1 mg/ml and measured again. Oligo B was diluted to 0.1 mg/ml and then measured.

The 0.1 mg/ml dilutions were also measured using the original SoloVPE System for comparison. The neat Oligo A samples were not tested on the SoloVPE System due to saturation of the detector at such high concentrations.

The 30 mg/ml sample of Oligo A was measured three times over two days. The remaining samples and concentrations were measured over the course of 3 days to assess the intermediate precision of the system and robustness of the method. The results were analyzed using the slope values to calculate the percent recovery using reference standards from Ionis. All samples were run in triplicate, and the results presented are based on the average values. The %RSD was calculated from the triplicate runs of each sample. Also recorded was the average R^2 value, which measures the linearity of the slope data and serves as an important requirement for compliance with the Beer-Lambert law.

Results and Discussion

Test results from the SoloVPE PLUS instrument are shown in Table 1. The system achieved greater than 96% recovery for all samples, and greater than 98% recovery for the 5 mg/ml and 0.1 mg/ml samples. The measurements demonstrated excellent repeatability, with less than 2% RSD for all samples and less than 0.5% RSD for the 5 mg/ml and 0.1 mg/ml samples. The R^2 value was consistently above the acceptance threshold of 0.999 for all measurements.

Table 1. Oligonucleotide measurement results - SoloVPE PLUS

Sample	Day	%Recovery	%RSD	Average R^2
Oilgo A, 30 mg/ml	1 (#1)	96.4%	1.20%	0.99989
	1 (#2)	97.6%	1.20%	0.99984
	2	97.8%	1.00%	0.99985
Oligo A, 5 mg/ml	1	99.9%	0.04%	0.99996
	2	99.8%	0.03%	0.99999
	3	99.9%	0.25%	0.99995
Oligo A, 0.1 mg/ml	1	98.8%	0.09%	1.00000
	2	98.3%	0.02%	1.00000
	3	98.2%	0.05%	1.00000
Oligo B, 0.1 mg/ml	1	98.8%	0.11%	1.00000
	2	98.5%	0.09%	1.00000
	3	98.3%	0.03%	1.00000

Intermediate precision test results are shown in Table 2. The %RSD was calculated from the slope values recorded across the three-day testing period. The SoloVPE PLUS System demonstrated high consistency for all samples, with less than 1% RSD over the three days.

Table 2. Intermediate precision results - SoloVPE PLUS

Sample	Day	Slope (Abs/mm)	%RSD
Oilgo A, 30 mg/ml	1 (#1)	68.519	0.53%
	1 (#2)	69.407	
	2	68.940	
Oligo A, 5 mg/ml	1	11.318	0.24%
	2	11.306	
	3	11.319	
Oligo A, 0.1 mg/ml	1	0.221	0.32%
	2	0.221	
	3	0.221	
Oligo B, 0.1 mg/ml	1	0.233	0.46%
	2	0.232	
	3	0.232	

Comparison data with the original SoloVPE System is shown in Table 3. All samples were measured with less than 1.2% difference between the two instruments, indicating excellent comparability.

Prior tests have shown that the SoloVPE System is limited to lower concentrations, while Tables 1 and 2 demonstrate the SoloVPE PLUS System's capability to read oligonucleotide concentrations up to 30 mg/ml. This increased limit of detection is achieved by the implementation of a stepper motor with a finer pathlength resolution, which can achieve higher slope values with minimal error.

Table 3. Comparability between SoloVPE and SoloVPE PLUS

Sample	Day	%Recovery, SoloVPE	%Recovery, SoloVPE PLUS	%Difference
Oligo A, 0.1 mg/ml	1	99.9%	98.8%	1.11%
	2	97.9%	98.3%	0.41%
	3	97.9%	98.2%	0.31%
Oligo B, 0.1 mg/ml	1	99.2%	98.8%	0.40%
	2	98.3%	98.5%	0.20%
	3	97.3%	98.3%	1.02%

Conclusion

This study demonstrated the SoloVPE PLUS System's capability to measure oligonucleotides with excellent accuracy and precision. The system achieved greater than greater than 98% recovery for samples up to 5 mg/ml, and greater than 96% recovery for the 30 mg/ml samples. Results were consistent across the three-day testing period, with less than 1% RSD between measurements on each day. The test also provided evidence of the SoloVPE PLUS System's improved dynamic range compared to the predecessor SoloVPE System, while maintaining comparable results. Overall, the SoloVPE PLUS System is a suitable instrument for rapid, accurate oligonucleotide measurement using the robust and reliable Slope Spectroscopy method.

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