CELL & GENE THERAPY INSIGHTS

RESEARCH ARTICLE

# Validation of mRNA concentration determination by slope spectroscopy technology: a matrixed approach

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mRNA-based therapeutics are different from small molecules and other biologics that represent significant analytical challenges. mRNA characterization for pre-clinical/clinical testing and lot release are required to compete in the competitive marketplace and align with regulatory standards. Faster and more reliable results require innovative solutions to meet these analytical challenges. Nucleic acid concentration determination is measured by determining the ultraviolet (UV) absorbances at an analytical wavelength of 260 nm. These absorbance measurements allow scientists to measure nucleic acid concentration based on the known extinction coefficient for RNA. The spectral signature of their maximum absorbance peak at 260 nm is proportional to nucleic acid concentration. The advantages of this UV nucleic acid quantitation method are that it is simple, direct, and requires just a small volume of your sample for measurement. One challenge, however, that the analytical labs run into is its limitation for specificity, as matrix components that absorb similar wavelengths can lead to inaccuracies in the consequent nucleic acid concentration determination. We have observed that the standard fixed-pathlength UV in current traditional cuvette-based UV solutions using a 1 cm cuvette and/or smaller fixed pathlengths still does not resolve the quality of the given measurement and lead to hours of required investigation time. The use of dilution factors, which increase prep time and variability, and fixed-pathlength measurements in determining the concentration of a UV chromophore in solution does not provide an easily transferable and robust method that can be platformed within a company or process. Today, researchers can selectively quantify nucleic acid absorbance in the presence of chemical and nucleic acid impurities, notably DNA and dsRNA. Analytical software uses full-spectrum data and advanced algorithms to identify nucleic acid impurities and provide corrected nucleic acid concentrations.

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## IMPROVING ANALYTICAL METHODS & TECHNIQUES

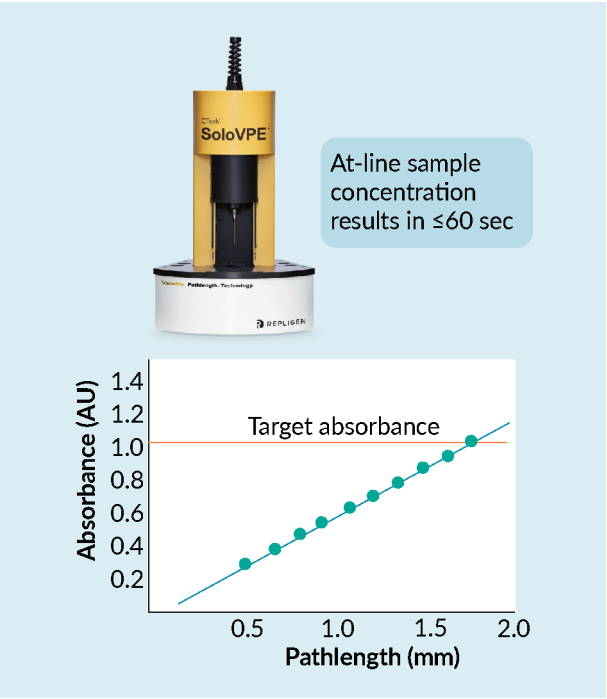
For cuvette-based spectrophotometry, sample handling and preparation (especially for in-process samples) creates challenges for bringing samples into the linear range of an instrument's detection capabilities. Additionally, performing serial dilutions for more complex sample matrices can have a significant impact on the calculated sample concentration due to pipetting accuracy and the risk of bubble introduction. As a result, the total assay variability may be larger than the acceptable range of concentration variance from the target value, calling into question the consistency of the method.

Unlike traditional UV-Vis methods that rely on a single absolute absorbance value, the slope spectroscopy method uses section data (absorbance versus pathlength data) to determine a slope value for quantitation of sample concentration using the slope spectroscopy equation ($m = \varepsilon c$) which is derived from the Beer–Lambert law (Figure 1). The $R^2$ value of the linear regression confirms that the absorbance values are changing proportionally when there is a change in absorbance over a change in pathlength following the Beer–Lambert law and therefore prove accuracy within every sample tested (Figure 2).

The CTech™ SoloVPE® variable pathlength spectrophotometer automatically adjusts the optical pathlength from 5µm–15mm in 5µm step increments. This provides the ability to determine the appropriate pathlength and linearity for significantly higher sample concentrations than those determined by fixed-pathlength spectrophotometers. The generated absorbance values are plotted into a linear slope regression with minimum $R^2$ of >0.999. Variable pathlength technology (VPT/slope spectroscopy) provides the speed, repeatability, and accuracy, to platform methods globally to eliminate the turnaround time and multiple personnel waiting for data results before continuing the next processing steps. As analytical testing capabilities continue

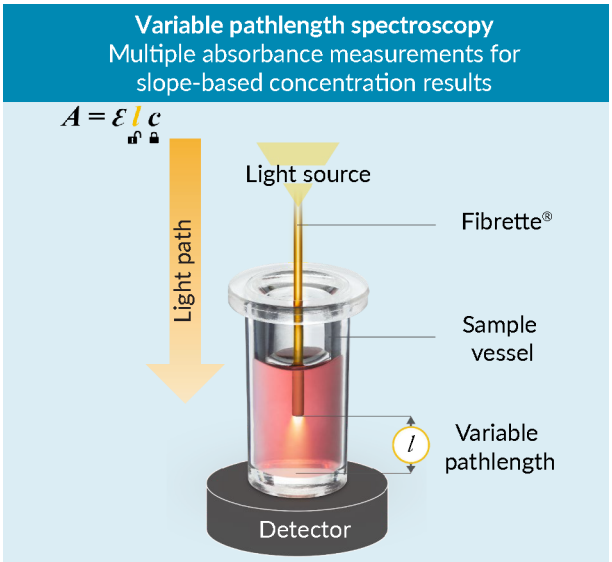
### FIGURE 1

**CTech™ SoloVPE® variable pathlength spectrophotometer.**




### FIGURE 2

**Variable pathlength spectroscopy.**





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to improve, these new technologies must be qualified and validated in accordance with regulatory guidance to ensure the highest level of product quality and patient safety. This white paper summarizes the strategy and results generated from a platform mRNA content assay for mRNA concentration.

## MATERIALS

- SoloVPE Instrument #1 [Part No. IN-SOLO5-VPE];
- SoloVPE Instrument #2 [Part No. IN-SOLO5-VPE];
- Cary 60 UV-Vis Spectrophotometer [IN-CARY60];
- Fibrette Optical Component [Part No. OF0002-P50];
- SoloVPE Sample Plastic Vessel [Part No. OC0009-P50];
- Solo Vessel Holder – Small [Part No. FA-CTIO1-PC26];
- Chem013 Measurement Standard [Part No. CHEM013-KIT];
- Tris-ethylenediaminetetraacetic acid (TE; 10 mM Tris, 1.0 mM EDTA, pH 8.0);
- Water for injection (WFI);
- 1 mM Sodium Citrate, pH 6.4;
- SpectraMax M5e Multimode Plate Reader;
- mRNA was produced in three representative sample matrices.

## STUDY DESIGN

This method was validated as a content assay as described in ICH Q2 (R1) [1]. The following validation parameters were assessed as part of the validation study: accuracy, repeatability, linearity, intermediate precision, specificity, and range. In addition, method comparability (e.g., bridging) to the platform cuvette-based UV spectrophotometry method was included to provide sufficient data to demonstrate method comparability to the current method.

The samples used in this validation study consisted of a single mRNA molecule formulated in the following sample matrices: TE (10 mM Tris, 1.0 mM EDTA, pH 8.0), Water for injection (WFI), and 1mM sodium citrate, pH 6.4. Each mRNA test sample was serially diluted two-fold in the appropriate sample matrix to generate a total of five concentration levels. All prepared validation samples were aliquoted into single use samples and stored at -80°C prior to validation. The mRNA concentration of Level 1 for each matrix was determined by UV-Spectrophotometry per the platform cuvette-based UV spectrophotometry method. The mean concentration result (mg/mL) from a total of three vials of Level 1 for each mRNA construct was determined and served as the target (theoretical) concentration for Level 1 for each sample matrix. Based on the UV established value of Level 1, the remaining levels' mRNA concentration was determined as the theoretical concentration following each two-fold dilution.

A single experiment was performed for accuracy, repeatability, linearity, specificity, and range by testing mRNA levels 1–5 of mRNA concentration. mRNA samples in TE were prepared at 4.10 mg/mL (L1), 2.05 mg/mL (L2), 1.03 mg/mL (L3), 0.51 mg/mL (L4), and 0.26 mg/mL (L5). mRNA samples in WFI were prepared at 4.70 mg/mL (L1), 2.3 mg/mL (L2), 1.18 mg/mL (L3), 0.59 mg/mL (L4), and 0.29 mg/mL (L5). mRNA samples in 1 mM sodium citrate, pH 6.4 were prepared at 4.01 mg/mL (L1), 2.01 mg/mL (L2), 1.00 mg/mL (L3), 0.50 mg/mL (L4), and 0.25 mg/mL (L5). Each level was tested over three analytical runs to generate three reportable results at each level. Sample matrix without the active ingredient was used as the sample for specificity and tested over a single analytical run. A second experiment

was performed for intermediate precision completed by a second analyst varying day and instrument. Method comparability (e.g., bridging) was evaluated by demonstrating acceptable accuracy (% recovery).

Prior to method validation, the non-interfering characteristics of the matrices were evaluated. Based on those development results (data not shown), corrections (baseline and/or scatter) were unnecessary and were not included in sample measurements. Concentration of a mRNA sample is determined by a modified Beer-Lambert Law Equation as described in C=m/ε.

Concentration (C, mg/mL) can be found by dividing the slope of the sample (m, Slope Abs (260 nm)/mm) by the known extinction coefficient (25 mL/(mg*cm)). Each mRNA sample was measured at both 260 nm and 280 nm.

## RESULTS

Validation results for accuracy are summarized in Tables 1–3. Relative accuracy (% recovery) was calculated for each mRNA level (1–5) for all mRNA constructs as described in the equation below using the measured mRNA concentration, and the theoretical mRNA concentration.

%Recovery= (measured mRNA Concentration)/(theoretical mRNA Concentation) × 100%

The % recoveries for all levels tested were well within the acceptance criterion of 85–115%.

Validation results for Repeatability are summarized in Tables 4–6. Repeatability was demonstrated by a single analyst over a total of three analytical runs by testing mRNA levels 1–5 for each sample matrix. %RSD was calculated for each level. The acceptance criterion of 10% RSD was met for all levels tested.

Validation results for Intermediate Precision are summarized in Tables 7–9. Intermediate precision of the method was demonstrated by calculating the % RSD of the combined results from two repeatability analytical runs completed by two analysts over 2 days and on separate instruments. The acceptance criterion of 10% RSD was met for all levels tested.

**TABLE 1**

**Accuracy results for TE sample matrix.**

| Matrix | Level | Analyst | Instrument | Vial | Theoretical concentration (mg/mL) | Measured concentration (mg/mL) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| TE | 1 | 1 | 1 | 6 | 4.10 | 4.25 | 104 |
| | | | | 11 | | 4.31 | 105 |
| | | | | 19 | | 4.28 | 104 |
| | 2 | 1 | 1 | 6 | 2.05 | 2.19 | 107 |
| | | | | 11 | | 2.20 | 107 |
| | | | | 19 | | 2.18 | 106 |
| | 3 | 1 | 1 | 6 | 1.03 | 1.09 | 106 |
| | | | | 11 | | 1.09 | 106 |
| | | | | 19 | | 1.09 | 107 |
| | 4 | 1 | 1 | 6 | 0.51 | 0.54 | 106 |
| | | | | 11 | | 0.54 | 106 |
| | | | | 19 | | 0.54 | 106 |
| | 5 | 1 | 1 | 6 | 0.26 | 0.27 | 106 |
| | | | | 11 | | 0.27 | 106 |
| | | | | 19 | | 0.27 | 107 |

TE: Tris-ethylenediaminetetraacetic acid.


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TABLE 2

**Accuracy results for WFI sample matrix.**

| Matrix | Level | Analyst | Instrument | Vial | Theoretical concentration (mg/mL) | Measured concentration (mg/mL) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| WFI | 1 | 1 | 2 | 6 | 4.70 | 4.58 | 97 |
| | | | | 11 | | 4.58 | 97 |
| | | | | 18 | | 4.62 | 98 |
| | 2 | 1 | 2 | 6 | 2.35 | 2.39 | 102 |
| | | | | 11 | | 2.40 | 102 |
| | | | | 18 | | 2.41 | 103 |
| | 3 | 1 | 2 | 6 | 1.18 | 1.22 | 104 |
| | | | | 11 | | 1.23 | 105 |
| | | | | 18 | | 1.22 | 104 |
| | 4 | 1 | 2 | 6 | 0.59 | 0.62 | 105 |
| | | | | 11 | | 0.62 | 106 |
| | | | | 18 | | 0.62 | 105 |
| | 5 | 1 | 2 | 6 | 0.29 | 0.31 | 107 |
| | | | | 11 | | 0.31 | 106 |
| | | | | 18 | | 0.31 | 106 |

WFI: Water for injection.

Validation results for Specificity are summarized in Table 10. Specificity was demonstrated by testing each mRNA matrix without analyte sample (Level 06) over a single analytical run completed by a single analyst. The acceptance criterion of 0.01 mg/mL or undetectable was met for all matrices.

Validation results for linearity are summarized in Tables 11–13 and are plotted in Figure 3. Linearity of the method was demonstrated by

TABLE 3

**Accuracy results for citrate sample matrix.**

| Matrix | Level | Analyst | Instrument | Vial | Theoretical concentration (mg/mL) | Measured concentration (mg/mL) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| Citrate | 1 | 1 | 1 | 6 | 4.01 | 3.80 | 95 |
| | | | | 11 | | 3.78 | 94 |
| | | | | 18 | | 3.81 | 95 |
| | 2 | 1 | 1 | 6 | 2.01 | 1.92 | 96 |
| | | | | 11 | | 1.91 | 95 |
| | | | | 18 | | 1.93 | 96 |
| | 3 | 1 | 1 | 6 | 1.00 | 0.96 | 96 |
| | | | | 11 | | 0.96 | 96 |
| | | | | 18 | | 0.96 | 96 |
| | 4 | 1 | 1 | 6 | 0.50 | 0.49 | 98 |
| | | | | 11 | | 0.49 | 98 |
| | | | | 18 | | 0.49 | 98 |
| | 5 | 1 | 1 | 6 | 0.25 | 0.24 | 96 |
| | | | | 11 | | 0.25 | 100 |
| | | | | 18 | | 0.25 | 100 |

TABLE 4

**Repeatability TE sample matrix.**

| Matrix | Level | Analyst | Vial | Measured concentration (mg/mL) | Average (mg/mL) | Standard deviation | RSD (%) |
|---|---|---|---|---|---|---|---|
| TE | 1 | 1 | 6 | 4.25 | 4.28 | 0.030 | 1 |
| | | 1 | 11 | 4.31 | | | |
| | | 1 | 19 | 4.28 | | | |
| | 2 | 1 | 6 | 2.19 | 2.19 | 0.010 | 0 |
| | | 1 | 11 | 2.20 | | | |
| | | 1 | 19 | 2.18 | | | |
| | 3 | 1 | 6 | 1.09 | 1.09 | 0.000 | 0 |
| | | 1 | 11 | 1.09 | | | |
| | | 1 | 19 | 1.09 | | | |
| | 4 | 1 | 6 | 0.54 | 0.54 | 0.000 | 0 |
| | | 1 | 11 | 0.54 | | | |
| | | 1 | 19 | 0.54 | | | |
| | 5 | 1 | 6 | 0.27 | 0.27 | 0.000 | 0 |
| | | 1 | 11 | 0.27 | | | |
| | | 1 | 19 | 0.27 | | | |

TE: Tris-ethylenediaminetetraacetic acid.

regression analysis of measured mRNA concentration (mg/mL) against the theoretical concentration (mg/mL) for mRNA levels 1–5 for each matrix. Linearity was assessed over three analytical runs completed by a single analyst. The coefficient of determination was determined as 1.00 for all matrices, which met the acceptance criterion of 0.98. The slope, and Y-intercept were also determined and are shown in Tables 11–13.

The working range of the method for mRNA samples in TE was established from

TABLE 5

**Repeatability WFI sample matrix.**

| Matrix | Level | Analyst | Vial | Measured concentration (mg/mL) | Average (mg/mL) | Standard deviation | RSD (%) |
|---|---|---|---|---|---|---|---|
| WFI | 1 | 1 | 6 | 4.58 | 4.59 | 0.023 | 1 |
| | | 1 | 11 | 4.58 | | | |
| | | 1 | 18 | 4.62 | | | |
| | 2 | 1 | 6 | 2.39 | 2.40 | 0.010 | 0 |
| | | 1 | 11 | 2.40 | | | |
| | | 1 | 18 | 2.41 | | | |
| | 3 | 1 | 6 | 1.22 | 1.22 | 0.006 | 0 |
| | | 1 | 11 | 1.23 | | | |
| | | 1 | 18 | 1.22 | | | |
| | 4 | 1 | 6 | 0.62 | 0.62 | 0.000 | 0 |
| | | 1 | 11 | 0.62 | | | |
| | | 1 | 18 | 0.62 | | | |
| | 5 | 1 | 6 | 0.31 | 0.31 | 0.000 | 0 |
| | | 1 | 11 | 0.31 | | | |
| | | 1 | 18 | 0.31 | | | |

RSD: Relative standard deviation; WFI: Water for injection.


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TABLE 6

**Repeatability citrate sample matrix.**

| Matrix | Level | Analyst | Vial | Measured concentration (mg/mL) | Average (mg/mL) | Standard deviation | RSD (%) |
|---|---|---|---|---|---|---|---|
| Citrate | 1 | 1 | 6 | 3.80 | 3.80 | 0.015 | 0 |
| | | 1 | 11 | 3.78 | | | |
| | | 1 | 18 | 3.81 | | | |
| | 2 | 1 | 6 | 1.92 | 1.92 | 0.010 | 1 |
| | | 1 | 11 | 1.91 | | | |
| | | 1 | 18 | 1.93 | | | |
| | 3 | 1 | 6 | 0.96 | 0.96 | 0.000 | 0 |
| | | 1 | 11 | 0.96 | | | |
| | | 1 | 18 | 0.96 | | | |
| | 4 | 1 | 6 | 0.49 | 0.49 | 0.000 | 0 |
| | | 1 | 11 | 0.49 | | | |
| | | 1 | 18 | 0.49 | | | |
| | 5 | 1 | 6 | 0.24 | 0.25 | 0.006 | 2 |
| | | 1 | 11 | 0.25 | | | |
| | | 1 | 18 | 0.25 | | | |

RSD: Relative standard deviation

4.10 to 0.26 mg/mL as passing results were obtained for linearity, accuracy, and precision. The working range of the method for mRNA samples in WFI was established from 4.70 to 0.29 mg/mL as passing results were obtained for linearity, accuracy, and precision. The

TABLE 7

**Intermediate precision results for TE sample matrix.**

| Matrix | Level | Analyst | Instrument | Vial | Measured concentration (mg/mL) | Average (mg/mL) | Standard deviation | RSD (%) |
|---|---|---|---|---|---|---|---|---|
| TE | 1 | 1 | 1 | 6 | 4.25 | 4.29 | 0.025 | 1 |
| | | 1 | 1 | 11 | 4.31 | | | |
| | | 1 | 1 | 19 | 4.28 | | | |
| | | 2 | 1 | 5 | 4.31 | | | |
| | | 2 | 1 | 15 | 4.30 | | | |
| | | 2 | 1 | 21 | 4.31 | | | |
| | 3 | 1 | 1 | 6 | 1.09 | 1.10 | 0.005 | 1 |
| | | 1 | 1 | 11 | 1.09 | | | |
| | | 1 | 1 | 19 | 1.09 | | | |
| | | 2 | 1 | 5 | 1.10 | | | |
| | | 2 | 1 | 15 | 1.10 | | | |
| | | 2 | 1 | 21 | 1.10 | | | |
| | 5 | 1 | 1 | 6 | 0.27 | 0.27 | 0.000 | 0 |
| | | 1 | 1 | 11 | 0.27 | | | |
| | | 1 | 1 | 19 | 0.27 | | | |
| | | 2 | 1 | 5 | 0.27 | | | |
| | | 2 | 1 | 15 | 0.27 | | | |
| | | 2 | 1 | 21 | 0.27 | | | |

RSD: Relative standard deviation; TE: Tris-ethylenediaminetetraacetic acid.

## TABLE 8

**Intermediate precision results for WFI sample matrix.**

| Matrix | Level | Analyst | Instrument | Vial | Measured concentration (mg/mL) | Average (mg/mL) | Standard deviation | RSD (%) |
|---|---|---|---|---|---|---|---|---|
| WFI | 1 | 1 | 2 | 6 | 4.58 | 4.60 | 0.026 | 1 |
| | | 1 | 2 | 11 | 4.58 | | | |
| | | 1 | 2 | 18 | 4.62 | | | |
| | | 2 | 2 | 5 | 4.64 | | | |
| | | 2 | 1 | 15 | 4.58 | | | |
| | | 2 | 1 | 21 | 4.59 | | | |
| | 3 | 1 | 2 | 6 | 1.22 | 1.22 | 0.004 | 0 |
| | | 1 | 2 | 11 | 1.23 | | | |
| | | 1 | 2 | 18 | 1.22 | | | |
| | | 2 | 2 | 5 | 1.22 | | | |
| | | 2 | 1 | 15 | 1.22 | | | |
| | | 2 | 1 | 21 | 1.22 | | | |
| | 5 | 1 | 2 | 6 | 0.31 | 0.31 | 0.000 | 0 |
| | | 1 | 2 | 11 | 0.31 | | | |
| | | 1 | 2 | 18 | 0.31 | | | |
| | | 2 | 2 | 5 | 0.31 | | | |
| | | 2 | 1 | 15 | 0.31 | | | |
| | | 2 | 1 | 21 | 0.31 | | | |

WFI: Water for injection.

## TABLE 9

**Intermediate precision results for citrate sample matrix.**

| Matrix | Level | Analyst | Instrument | Vial | Measured concentration (mg/mL) | Average (mg/mL) | Standard deviation | RSD (%) |
|---|---|---|---|---|---|---|---|---|
| Citrate | 1 | 1 | 1 | 6 | 3.80 | 3.79 | 0.013 | 0 |
| | | 1 | 1 | 11 | 3.78 | | | |
| | | 1 | 1 | 18 | 3.81 | | | |
| | | 2 | 1 | 5 | 3.78 | | | |
| | | 2 | 2 | 15 | 3.78 | | | |
| | | 2 | 2 | 21 | 3.79 | | | |
| | 3 | 1 | 1 | 6 | 0.96 | 0.96 | 0.000 | 0 |
| | | 1 | 1 | 11 | 0.96 | | | |
| | | 1 | 1 | 18 | 0.96 | | | |
| | | 2 | 1 | 5 | 0.96 | | | |
| | | 2 | 2 | 15 | 0.96 | | | |
| | | 2 | 2 | 22 | 0.96 | | | |
| | 5 | 1 | 1 | 6 | 0.24 | 0.25 | 0.005 | 2 |
| | | 1 | 1 | 11 | 0.25 | | | |
| | | 1 | 1 | 18 | 0.25 | | | |
| | | 2 | 1 | 5 | 0.24 | | | |
| | | 2 | 2 | 15 | 0.24 | | | |
| | | 2 | 2 | 21 | 0.25 | | | |

RSD: Relative standard deviation.


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## TABLE 10

**Validation specificity results.**

| Matrix | Level | Analyst | Vial | Theoretical concentration (mg/mL) | Measured concentration (mg/mL) |
|---|---|---|---|---|---|
| TE | 06 | 1 | 6 | 0 (matrix only) | 0.00 |
| WFI | | | 6 | | 0.00 |
| Citrate | | | 6 | | 0.00 |

TE: Tris-ethylenediaminetetraacetic acid; WFI: Water for injection.

## TABLE 11

**Linearity results for TE sample matrix.**

| Level | Average measured concentration (mg/mL) | Theoretical concentration (mg/mL) | Validation criterion |
|---|---|---|---|
| 1 | 4.30 | 4.10 | $R^2$ is ≥ 0.98 |
| 2 | 2.19 | 2.05 | |
| 3 | 1.09 | 1.03 | |
| 4 | 0.54 | 0.51 | |
| 5 | 0.27 | 0.26 | |
| Identifier | | Result | |
| Coefficient of determination (R2) | | 1.00 | |
| Identifier | | Results (report only) | |
| Slope | | 1.045 | |
| Y-intercept | | 0.01 | |

TE: Tris-ethylenediaminetetraacetic acid.

## TABLE 12

**Linearity results for WFI sample matrix.**

| Level | Average measured concentration (mg/mL) | Theoretical concentration (mg/mL) | Validation criterion |
|---|---|---|---|
| 1 | 4.59 | 4.70 | $R^2$ is ≥ 0.98 |
| 2 | 2.40 | 2.35 | |
| 3 | 1.22 | 1.18 | |
| 4 | 0.62 | 0.59 | |
| 5 | 0.31 | 0.29 | |
| Identifier | | Result | |
| Coefficient of determination (R2) | | 1.00 | |
| Identifier | | Results (report only) | |
| Slope | | 0.97 | |
| Y-intercept | | 0.06 | |

WFI: Water for injection.

working range of the method for mRNA samples in 1 mM sodium citrate, pH 6.4 was established from 4.01 to 0.25 mg/mL as passing results were obtained for linearity, accuracy, and precision.

Robustness of the method was evaluated as part of method development. Robustness – sample volume robustness (100 and 140 L) was demonstrated by a single analyst over a total of three analytical runs by testing

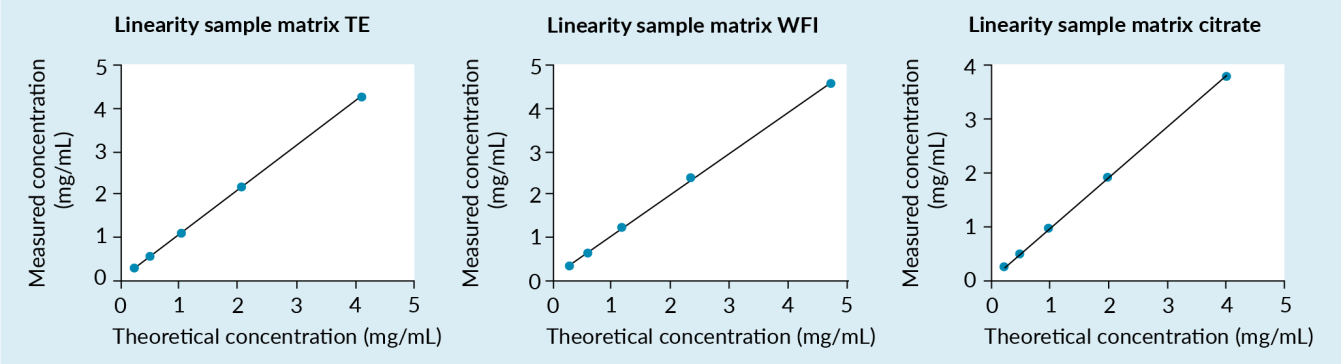
## TABLE 13

**Linearity results for citrate sample matrix.**

| Level | Average measured concentration (mg/mL) | Theoretical concentration (mg/mL) | Validation criterion |
|---|---|---|---|
| 1 | 3.80 | 4.01 | $R^2$ is ≥ 0.98 |
| 2 | 1.92 | 2.01 | |
| 3 | 0.96 | 1.00 | |
| 4 | 0.49 | 0.50 | |
| 5 | 0.25 | 0.25 | |
| Identifier | | Result | |
| Coefficient of determination (R2) | | 1.00 | |
| Identifier | | Results (report only) | |
| Slope | | 0.94 | |
| Y-intercept | | 0.02 | |

## FIGURE 3

**Linear regression analysis (A–C) for each sample matrix.**




Data are reported as mean ± SEM).

## TABLE 14

**Robustness–sample volume results.**

| Matrix | Level | Vial | Volume (L) | Measured concentration (mg/mL) | % difference |
|---|---|---|---|---|---|
| TE | 1 | 4 | 100 | 4.32 | 2 |
| | | 12 | 100 | 4.37 | 1 |
| | | 22 | 100 | 4.34 | 1 |
| | | 4 | 140 | 4.35 | 1 |
| | | 12 | 140 | 4.33 | 2 |
| | | 22 | 140 | 4.33 | 2 |

TE: Tris-ethylenediaminetetraacetic acid.
Note: The % difference is calculated from the measured concentration (mg/mL) of the 120-L sample volume.
Note: Theoretical concentration was 4.10 mg/mL.

mRNA levels 1, 3, and 5 for a single mRNA sample matrix. Percent difference from the measured mRNA result obtained in Accuracy (tested at 120 L) was calculated for each level. Results are summarized in Tables 14–16. These data indicate that the method is relatively unaffected by slight variations in sample volume.

Robustness–sample mixing of the method was demonstrated by a single analyst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over a total of three analytical runs by testing mRNA levels 1, 3, and 5 for a single mRNA matrix. Percent difference from the measured mRNA result obtained in accuracy was calculated for each level. Results are summarized in Table 17. These data indicate that the method is relatively unaffected by these variations in sample mixing.

Bridging between the platform cuvette-based UV spectrophotometry method was confirmed as the acceptance criterion for accuracy was met.

## TABLE 15

**Robustness–sample volume results.**

| Matrix | Level | Vial | Volume (L) | Measured concentration (mg/mL) | % difference |
|---|---|---|---|---|---|
| TE | 3 | 4 | 100 | 1.10 | 2 |
| | | 12 | 100 | 1.11 | 1 |
| | | 22 | 100 | 1.11 | 1 |
| | | 4 | 140 | 1.09 | 2 |
| | | 12 | 100 | 1.11 | 1 |
| | | 22 | 140 | 1.10 | 2 |

TE: Tris-ethylenediaminetetraacetic acid.
Note: The % difference is calculated from the measured concentration (mg/mL) of the 120-L sample volume.
Note: Theoretical concentration was 1.03 mg/mL.

## TABLE 16

**Robustness–sample volume results.**

| Matrix | Level | Vial | Volume (L) | Measured concentration (mg/mL) | % difference |
|---|---|---|---|---|---|
| TE | 5 | 4 | 100 | 0.27 | 3 |
| | | 12 | 100 | 0.27 | 2 |
| | | 22 | 100 | 0.28 | 1 |
| | | 4 | 140 | 0.27 | 4 |
| | | 12 | 140 | 0.27 | 3 |
| | | 22 | 140 | 0.27 | 3 |

TE: Tris-ethylenediaminetetraacetic acid.
Note: The % difference is calculated from the measured concentration (mg/mL) of the 120-L sample volume.
Note: Theoretical concentration was 0.29 mg/mL.

## TABLE 17

**Robustness–sample volume results.**

| Matrix | Level | Mixing condition | Vial | Measured concentration (mg/mL) | % difference |
|---|---|---|---|---|---|
| WFI | 3 | Hand inversion | 4 | 1.21 | 0 |
| | | | 14 | 1.23 | 2 |
| | | | 22 | 1.21 | 1 |
| | | Aggressive vortex | 4 | 1.20 | 0 |
| | | | 14 | 1.19 | 1 |
| | | | 22 | 1.23 | 2 |

WFI: Water for injection.
Note: The % difference is calculated from the measured concentration (mg/mL) of the 120-L sample volume.

## CONCLUSION

These data demonstrate that the SoloVPE variable pathlength spectrophotometer is a preferable alternative to traditional UV spectrophotometry. The versatility of variable pathlength technology (VPT/slope spectroscopy) is impactful for a Contract Development and Manufacturing Organization (CDMO), as it allows for the development and validation of platform analytical methods, resulting in many advantages over traditional UV spectrophotometry.

The challenge for CDMOs is the complexity in developing and validating analytical methods that can support several clients all with unique constructs. The SoloVPE technology, combined with a comprehensive platform validation covering multiple concentrations and formulation buffers, provides great benefits to CDMO clients and ensures the quality and the consistency of the CDMO's products for the reasons highlighted below:

- The SoloVPE technology is platformable. A single test method can be leveraged to support multiple mRNA products with no need to redevelop and validate multiple platform analytical methods to test mRNA concentration. The versatility of the SoloVPE reduces the cycle time for product-specific work, helping to support streamlined product release;
- The SoloVPE is accurate and repeatable. In contrast to traditional cuvette-based UV spectroscopy which relies on a single data point measured from the sample to determine the concentration, SoloVPE measures multiple data points from the sample at several pathlengths to determine the concentration. In addition, sample handling is effectively eliminated as no dilution of the test sample is required;
- The SoloVPE is easy to use. Complex methodologies can introduce error leading to unnecessary investigations and delays in product release. SoloVPE reduces method complexity while delivering a technology that is extremely robust and well suited for a release laboratory setting.

Onboarding new technologies like the SoloVPE allows CDMOs to continue to provide comprehensive analytical testing capabilities to support their clients' needs.

## REFERENCE

1. ICH Q2 (R1) Validation of Analytical Procedures.

## BIOGRAPHIES

**TRAVIS ALVINE** leads the analytical method validation group and manages analytical method validation, method transfer, and method implementation into the Quality Control release laboratory at Aldevron's Fargo location. He has been with Aldevron for over 6 years, with experience in analytical method development and validation over that time.

**NATALIE UNSINN** operates within the analytical method validation team through validation of methods, collaboration across sites, and implementation of methods to the quality control release team. She has been with Aldevron for over 3 years, with experience in quality control and analytical method development and validation.

**JOHN J LONG** has over 20 years of experience working in analytical methods for large molecule therapeutics including Vaccines, Biologics, and Gene Therapy products. He has experience across the analytical life cycle including method development, validations, and transfers worldwide. John has


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supported products from early development through licensure as well as support for marketed products. He has broad experience in compliance, investigations, inspections, and regulatory submissions.

**JOE FERRAIOLO** leads the bioanalytics applications team and is in charge of the SoloVPE variable pathlength spectroscopy system for at-line applications. He has been with the company for more than 25 years, with over 15 years of development and validation experience in analytical applications. He specializes in UV analysis and leads the development and commercialization of high-value products and flexible solutions that address critical steps in the production of biologics.


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## AUTHORSHIP & CONFLICT OF INTEREST

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