A Universal Assay Determination Method for Antisense Oligonucleotides

A New Approach Using Slope Spectroscopy

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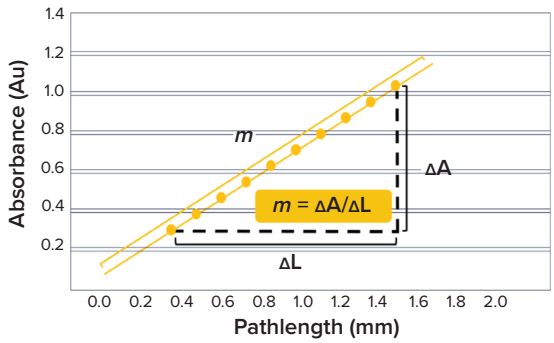
Antisense oligonucleotides (ASOs) are short, synthetic, single-stranded oligodeoxynucleotides that can alter RNA and reduce, restore, or modify protein expression through several distinct mechanisms. ASO technology has become an important drug discovery platform for most major pharmaceutical companies. To date, six antisense drugs have been approved by regulatory agencies to treat diseases spanning viral infections, hyperlipidemias, and neurological diseases. Well over 50 additional ASO drugs are in various stages of clinical trials.

For an ASO drug product, an assay of its active pharmaceutical ingredient (API) is a critical quality attribute (CQA) because of its direct impact on drug safety and efficacy. Therefore, proper in-process control (IPC) measurements must be implemented to ensure that an ASO assay is within an acceptable range during different unit operations of drug product manufacturing (e.g., API compounding and bulk filtration). We have used a platform HPLC-UV method for IPC assay measurements. Recently, we have developed a SoloVPE system assay method using slope spectroscopy that can provide both accuracy and precision comparable with those of HPLC. Moreover, the method also provides several appealing advantages over use of HPLC, including simpler instrument setup, test procedure, and data analysis, all of which contribute to a shorter turnaround time.

This application note demonstrates the SoloVPE System's universal ability to measure assays for ASOs of different chemical modifications precisely and accurately (see the Materials and Equipment box). We consider the slope spectroscopy method to be a promising new platform IPC assay method for ASO drug products.



Method Principle

The determination of ASO concentration using the SoloVPE system is based on the following Beer-Lambert law–derived slope-spectroscopy equation:

$$m = \varepsilon c$$

where m is the slope of the regression line by plotting absorbance as a function of pathlength at a given wavelength, ε is the extinction coefficient, and c is the sample concentration. Our method uses an ASO reference standard solution of a known concentration to eliminate a need to predetermine the ε value of the ASO. In this case, the sample concentration can be calculated using the following equation:

Materials and Equipment

SoloVPE device, C Technologies, Inc. a Repligen Company

Cary 60 UV-vis spectrophotometer, Agilent Technologies

ConfiRM–MID (m0.14) slope standard, C Technologies catalog #MRM-07-P10

SoloVPE Fibrette optic cable, C Technologies catalog #OF0002-P50

Larges fused silica vessel (15 mm) C Technologies catalog #OC0005-2

ASO GMP and development batches (various lots), Ionis Pharmaceuticals

ASO reference standard solutions (various lots), Ionis Pharmaceuticals

$$C_{Sample} = \frac{m_{Sample}}{m_{Standard}} C_{Standard}$$

where $C_{Standard}$ and C_{Sample} are concentrations of the standard and the sample, respectively, and $m_{Standard}$ and m_{Sample} are slopes of the standard and the sample at 260 nm, respectively. The measured sample concentration is

further adjusted by an API purity factor to be reported as the final assay value.

Results and Discussion

The SoloVPE system method was extensively tested for ASOs with a number of chemistry modifications including but not limited to base modifications, sugar modifications, internucleoside linkage modifications, and *N*-acetylgalactosamine (GalNAc) conjugates. Furthermore, the method has been successfully validated internally as well as at contract manufacturing organizations (CMOs) for a number of ASOs in accordance with ICH Q2(R1) guidance.

Table 1 shows repeatability and accuracy results of representative ASOs in different aqueous solutions. An expected assay of each sample was measured by a qualified LC-UV-MS method. In general, slope %RSD is no more than 0.2% from triplicate measurements for all the studied ASOs, whereas assay %recovery is within 98.0%–102.0% when compared with the expected value.

Figures 1 and 2 show linearity plots for ASO_B and ASO_D: variations of mean slope as a function of ASO concentration ranging from 50% to 150% of the nominal target value and corresponding linear regression fitting curves by setting the intercept to 0. The excellent fit (R^2 = 0.9989 for ASO_B and R^2 = 0.9992 for ASO_D) confirmed the validity of the linear equation described in the above method principle.

Table 2 shows intermediate precision results for one sample preparation of ASO_B measured by two different analysts on three different test days. We saw excellent intermediate precision with both interday %RSD and overall %RSD <1.0%.

Robustness was tested further on ASO_B, as shown in Table 3. When running this test, we made some deliberate deviations from the standard operating procedure. Among all those modifications to the method, only the action of leaving the sample cover opened led to a significant increase in slope %RSD and an obviously underestimated assay value. This increased data variability can be explained by stray light affecting the measurement with the instrument cover open, leading to out-of-specification results. Those can be remedied easily if the same sample is remeasured with the cover properly closed. Whereas this particular test scenario failed the robustness criteria as expected, all other tested protocol deviations passed

Figure 1: Linearity plot for ASO_B.

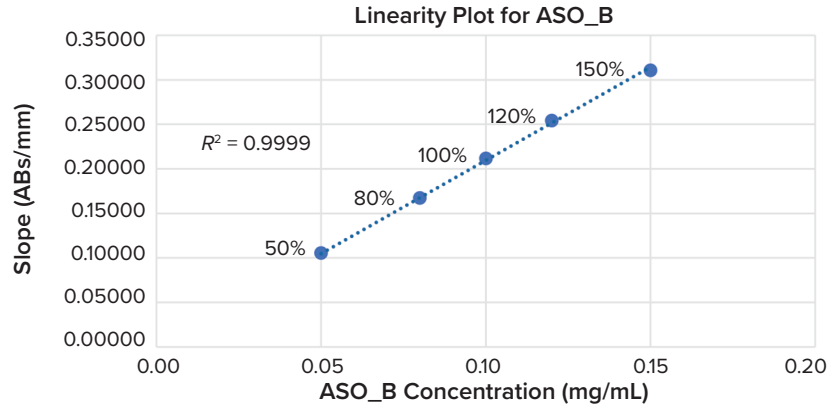


Figure 2: Linearity plot for ASO_D.

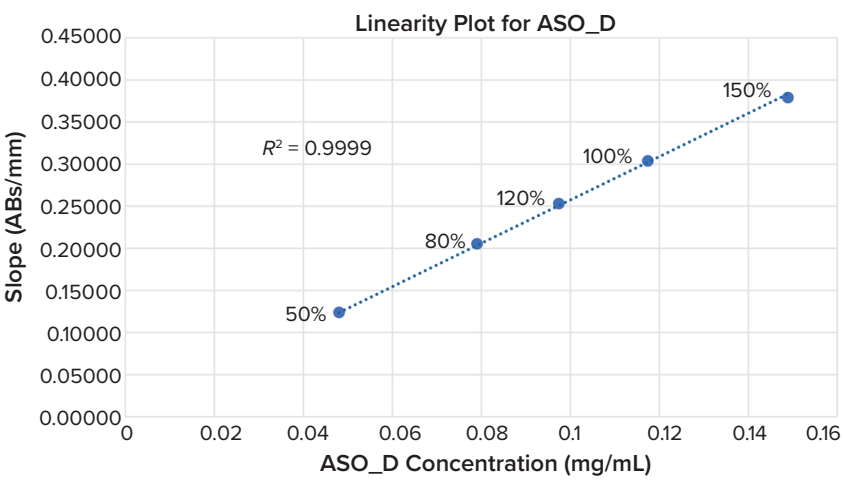


Table 1: Repeatability and accuracy results

API	Vehicle	Sample Preparation Concentration	Mean Slope* (Abs/mm)	Slope %RSD*	Measured Assay (mg/mL)	Expected Assay (mg/mL)	Assay %Recovery
ASO_A	Buffer X	50% Nominal	0.10912	0.0%	151.2	149.3	101.3%
		100% Nominal	0.21054	0.0%	151.2		101.3%
		150% Nominal	0.32707	0.1%	150.8		101.0%
ASO_B	Buffer Y	50% Nominal	0.11111	0.2%	56.66	56.70	99.9%
		100% Nominal	0.21839	0.1%	56.57		99.8%
		150% Nominal	0.32786	0.1%	56.54		99.7%
ASO_C	Buffer Z	50% Nominal	0.11575	0.2%	19.96	19.78	100.9%
		100% Nominal	0.23115	0.1%	19.95		100.9%
		150% Nominal	0.34567	0.2%	19.94		100.8%
ASO_D	Buffer Z	50% Nominal	0.12756	0.0%	19.69	19.54	100.8%
		100% Nominal	0.24838	0.0%	19.61		100.4%
		150% Nominal	0.37438	0.0%	19.64		100.5%
ASO_E	Buffer Z	50% Nominal	0.12838	0.1%	19.58	19.54	100.2%
		100% Nominal	0.25260	0.0%	19.55		100.1%
		150% Nominal	0.37929	0.1%	19.62		100.4%

* From triplicate measurements

Table 2: Intermediate precision results for ASO_B

Analyst	Test Day	Assay %Recovery	Inter-Day Mean Assay %Recovery	Inter-Day %RSD	Inter-Analyst and Inter-Test Day Mean Assay %Recovery	Inter-Analyst and Inter-Test Day %RSD
1	1	100.6%	100.5%	0.4%	100.4%	0.3%
	2	100.1%				
	3	100.8%				
2	1	100.5%	100.3%	0.3%		
	2	100.0%				
	3	100.2%				

Table 3: Method robustness results for ASO_B

Adjustment	Mean Slope* (Abs/mm)	Slope %RSD*	Measured Assay (mg/mL)	Assay %Recovery	%[difference] to No Admustment
No adjustment	0.21731	0.1%	56.30	99.3%	—
Reused Fibrette	0.21837	0.3%	56.66	99.8%	0.5%
No Sample Holder	0.21911	0.1%	56.76	100.1%	0.8%
Sample Cover Opened	0.21048	5.5%	54.49	96.1%	3.2%
No Fibrette Drop Down	0.22075	0.1%	56.76	100.1%	0.8%
Wavelength Shift Nominal +2 nm	0.21706	0.3%	56.42	99.5%	0.2%
Wavelength Shift Nominal −2 nm	0.21583	0.1%	56.64	99.9%	0.6%
30 min hold time in sample vessel	0.22104	0.3%	56.87	100.3%	1.0%

* From triplicate measurements

Table 4: Method reproducibility results

API	Sample Preparation	Sample Assay %Recovery In-house	Sample Assay %Recovery CMO X	Sample Assay %Recovery CMO Y	[difference] Internal vs. CMO
ASO_A	50% Nominal	101.4%	101.6%	—	0.2%
	100% Nominal	101.2%	101.8%		0.6%
	150% Nominal	101.1%	102.0%		0.9%
ASO_B	50% Nominal	99.9%	99.4%		0.5%
	100% Nominal	99.8%	99.7%		0.1%
	150% Nominal	99.7%	99.9%		0.2%
ASO_C	50% Nominal	100.9%	—	99.2%	1.7%
	100% Nominal	100.9%		99.5%	1.4%
	150% Nominal	100.8%		99.4%	1.4%
ASO_D	50% Nominal	100.8%		100.9%	0.1%
	100% Nominal	100.4%		101.2%	0.8%
	150% Nominal	100.5%		100.6%	0.1%

with a very low %RSD of 0.1–0.3% and no more than 1% difference from the standard protocol, demonstrating the excellent robustness of the method.

This method has been transferred successfully to CMOs and implemented in GMP drug-product manufacturing. Table 4 shows method reproducibility results of four different ASOs at the CMOs. Good reproducibility of the method between in-house and CMO results was confirmed, with the difference in assay %recovery less than 2.0% for all the tested samples.

Accurate, Precise, and Robust

The SoloVPE System assay method has been proven to be accurate, precise, and robust. Therefore, it can easily and successfully be validated in accordance with ICH Q2(R1) requirements for chemically modified ASOs. We have shown herein that the slope spectroscopy method can be implemented universally across multiple sites with consistent results throughout product transfers. For all products tested for percent recovery, this resulted in an overall difference of <2%. Our results highlight the simplicity and performance of the SoloVPE system technology in comparison with HPLC. The results provide the same level of accuracy and precision, but the overall time savings are greatly increased. Using the former method would take approximately six hours, whereas by using the SoloVPE system, multiple samples can be measured in under two hours, providing a 67% cost/time reduction compared with HPLC. The slope spectroscopy method enables significant process improvements that decrease turnaround time and simplify testing procedures. Therefore, the SoloVPE system and the slope spectroscopy method are qualified to serve as a more efficient and universal IPC assay instrument and method for AS0 drug-product manufacturing.

For Further Reading

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****This citation is for a one-page erratum from the full publication in 2018 – is this the only page you want to call attention to, or shall I list the original issue details?****

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