Application Note

Pharma

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Ethanol Impurity Analysis Using the Agilent Cary 3500 Flexible UV-Vis Spectrophotometer

The use of long-path-length cells in pharmaceutical QC

Author

Chloe Hsieh
Agilent Technologies, Inc.

Abstract

Ethanol used in pharmaceutical manufacturing must be of high purity and comply with pharmacopeia standards. This application note explores the use of an Agilent Cary 3500 Flexible UV-Vis spectrophotometer and a variable long-path-length holder to verify the purity levels of two ethanol samples in accordance with the US, European, and Japanese Pharmacopoeia monographs for ethanol. Of the two ethanol samples measured, the 96% undenatured ethanol sample passed, while the 100% undenatured ethanol sample did not pass the acceptance criteria for impurity absorbance. This application note also highlights how Agilent Cary UV Workstation software enables the creation of simple, efficient processes for routine quality checks.

Introduction

Ultraviolet-visible (UV-Vis) spectroscopy is a rapid, sensitive, and nondestructive test widely used in the pharmaceutical industry for quality control (QC). This method typically involves using a standard 10 mm path length cuvette to measure absorbance of materials, which can then be used in QC labs to quantify or qualify an analyte of interest.

In UV-Vis measurements, the photometric response of the spectrophotometer follows the Beer-Lambert law, where a linear relationship exists between the sample absorbance and sample path length. The path length is the distance that the incident light travels through a sample. Increasing the cuvette path length from 10 to 50 mm increases the absorbance of a sample, aiding in more accurate quantification of components with low peak intensity. Alternatively, if the sample concentration is high and exceeds the linear dynamic detection range of the system, a cuvette with a shorter path length can be used instead.[1]

In the pharmaceutical industry, ethanol is widely used in manufacturing as a disinfectant, solvent, and preservative. Due to its amphipathic structure, ethanol can dissolve both hydrophilic and lipophilic substances, serving as a medium to dissolve key components for a broad spectrum of drug formulations. The dual nature of ethanol also allows it to inhibit microbial growth by disrupting cellular membranes and denaturing proteins, processes that are vital for disinfection and the preservation of medications.[2]

As a result, ethanol used in pharmaceutical manufacturing must comply with pharmacopeia standards to ensure drug safety, efficacy, and quality. The United States Pharmacopeia (USP)[3], European Pharmacopoeia (EP)[4], and Japanese Pharmacopoeia (JP)[5] all specify using an ultraviolet absorption test to measure impurities in ethanol at designated wavelengths (Table 1). In this test, the absorption spectrum should show a steadily descending curve with no observable peaks or shoulders, which serve as qualitative indicators of impurities present in the sample. Since the impurities have low absorbance levels, the USP, EP, and JP require the use of a 50 mm cell to improve sensitivity and detection levels.

Table 1. Absorbance limits for impurities in ethanol based on the three pharmacopeias (USP, EP, and JP).

	Wavelength (nm)		
	240	250–260	270–340
Absorbance (Abs)	No more than 0.40	No more than 0.30	No more than 0.10

This application note demonstrates the use of a 50 mm path length cell holder with the Agilent Cary 3500 Flexible UV-Vis spectrophotometer (Figure 1) and the Agilent Cary UV Workstation software to easily evaluate ethanol quality in accordance with the USP, EP, and JP monographs for ethanol.

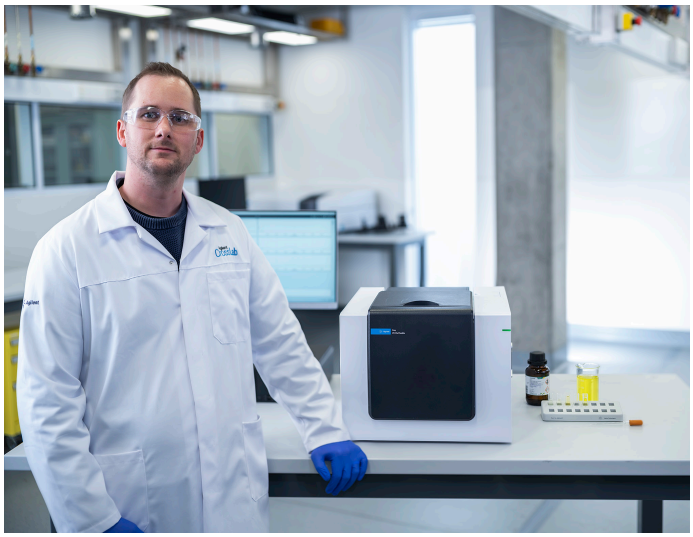

Figure 1. The Agilent Cary 3500 Flexible UV-Vis spectrophotometer, which supports liquid sample measurements at different path lengths up to 10 cm using toolless variable-path-length rectangular and cylindrical cell holders.

Experimental

Sample preparation

Blank: The blank was prepared by transferring 17 mL of Milli-Q water (Millipore, USA) into an Agilent 50 mm path length rectangular quartz cuvette (part number 6610016100).

Samples: Two samples of commercially available ethanol (Chem-Supply, part number EL043-20L-P, 100% undenatured, CAS no. 64-17-5; and Chem-Supply, part number EA042-2.5L-P, 96% undenatured, CAS no. 64-17-5) were prepared by transferring 17 mL of each into separate 50 mm path length quartz rectangular cuvettes (part number 6610016100). The cuvettes were capped with a lid to minimize evaporation.

Instrumentation: Once the samples were prepared, their absorbances were measured using the Cary 3500 Flexible UV-Vis spectrophotometer and the variable long-path-length cell holder set to 50 mm. The instrument parameters used for these measurements are listed in Table 2.

Table 2. Data collection parameters for measurements on the Agilent Cary 3500 Flexible UV-Vis spectrophotometer.

Parameter	Setting
Wavelength Range	235–340 nm
Signal Averaging Time	0.1 s
Data Interval	1.00 nm
Spectral Bandwidth	2.00 nm
Blank	Water
Accessory	Long-path-length cell holder set to 50 mm

Results and discussion

The customizable calculator in the Cary UV Workstation software was used to evaluate absorbance values for the ethanol samples at the wavelengths specified in the US, European, and Japanese Pharmacopoeias, which define the acceptable impurity absorbance limits. Using Scan batch, the UV-Vis spectra of ethanol samples were collected across the 235–340 nm wavelength range, and the shapes of the absorbance curves were observed. The "end-of-sequence analysis" feature was used to automatically identify the maximum absorbance within the specified wavelength ranges and determine whether the ethanol samples passed the acceptance criteria (Figure 2).

Once the baseline was measured using water (blank), the Cary UV Workstation software produced consecutive spectra for the two ethanol samples and a table of absorbance values based on the end-of-sequence analysis setup in Figure 2.

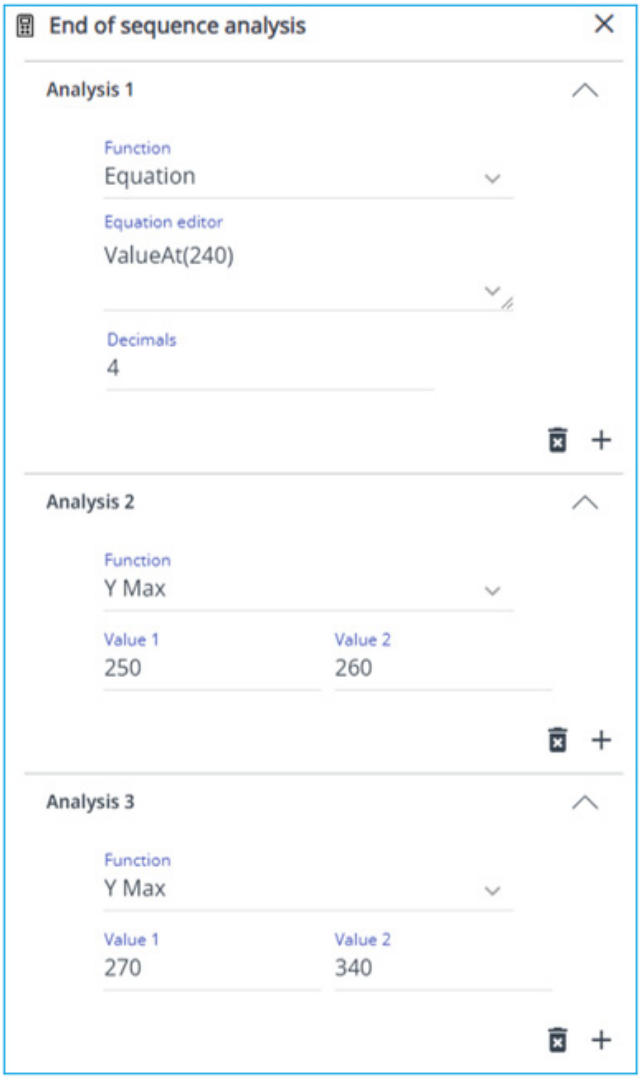

Figure 2. Built-in "end-of-sequence analysis" feature in the Agilent Cary UV Workstation software used for ethanol evaluation. The calculator provides flexibility to create custom equations using sample metadata, which can be saved within the method to improve efficiency and minimize errors.

As shown in Figure 3, the 100% undenatured ethanol (blue curve) passed two of the three absorbance criteria, exceeding only the threshold of 0.4 at 240 nm. However, the absorbance spectrum for this sample displays a smooth, descending curve with no visible bumps or shoulders, suggesting the presence of trace impurities rather than contamination or general impurities. Although the 96% undenatured ethanol (red curve) passed all three absorbance criteria, its spectrum shows a steadily descending curve with a bump in the 260–290 nm range. This bump suggests the presence of impurities that fall within the acceptable limit for pharmaceutical applications. As the 96% denatured ethanol sample met all three absorbance criteria dictated in the US, European, and Japanese Pharmacopoeias, it is suitable for pharmaceutical applications, unlike the 100% denatured ethanol sample.

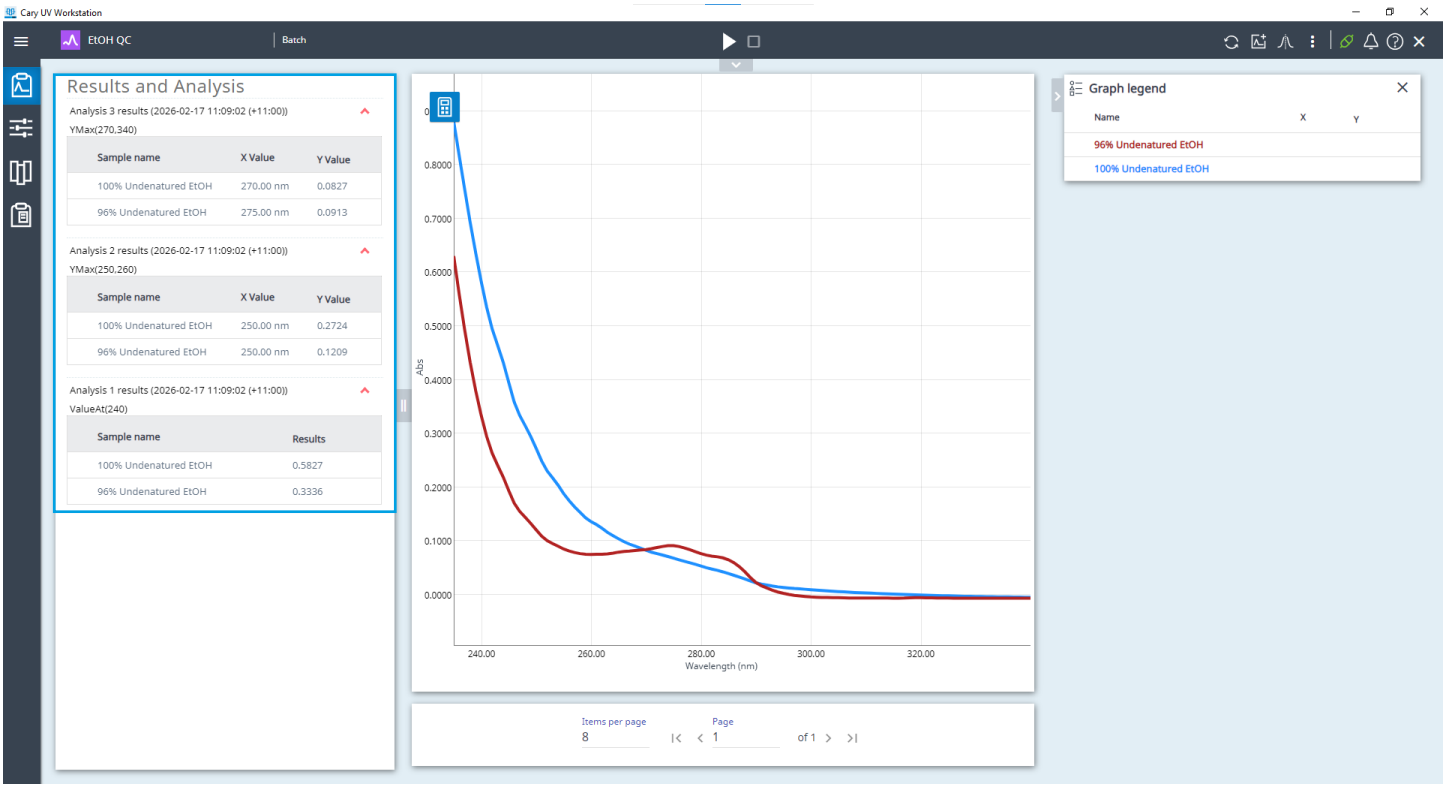

Figure 3. Results and analysis interface in Agilent Cary UV Workstation software after measurement. The results are automatically populated in a table at the end of the analysis, as highlighted in the blue box.

Report generation

In the pharmaceutical industry, reporting must be accurate and traceable for reproducibility and compliance. The Cary UV Workstation software facilitates this through an integrated Agilent OpenLab software package featuring administrative controls, including audit trails, to ensure that data is securely acquired and stored in full compliance with FDA 21 CFR Part 11 and EU Annex 11.[6]

After measurement, the software automatically generates scan reports (in PDF and/or CSV format), consolidating essential information such as instrument parameters, sample information, and calculated parameters—all tailored to user preferences (Figure 4). These key parameters can then be saved as a method in the Cary UV Workstation software, enabling future quality checks for ethanol purity to be streamlined efficiently and eliminating the need to manually set up sequencing parameters for each analysis.

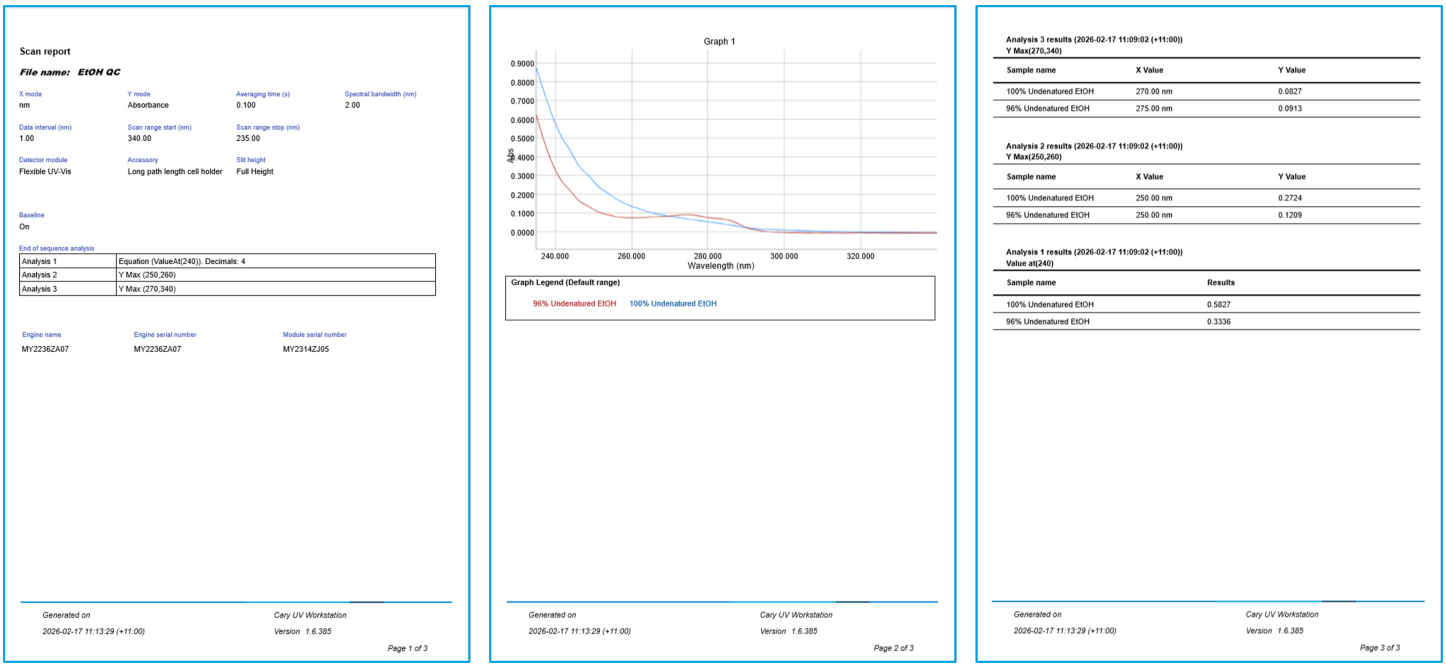

Figure 4. Scan report generated by Agilent Cary UV Workstation software. The instrument parameters and end-of-sequence analysis calculations are saved as a method, enabling simple and time-saving analysis for future routine use.

A reliable system with better data integrity

For analyses that require the use of a longer-path-length cell, the Cary 3500 Flexible module features a unique variable-path-length holder suitable for rectangular and cylindrical cells.[1] The holder includes built-in notches that enable the user to quickly and reproducibly change the path length between 20, 40, 50, and 100 mm without any tools, eliminating the time-consuming alignment procedure (Figure 5).

Traditional UV-Vis lamps, such as deuterium or tungsten, must be replaced annually, which is a time-consuming and costly process. The Agilent Cary 3500 Flexible UV-Vis spectrophotometer is equipped with a robust xenon flash lamp, eliminating warm-up time delays and saving replacement costs. The lamp also comes with a 10-year warranty.

Additionally, prior to any analysis using a UV-Vis spectrophotometer, it is critical for pharmaceutical laboratories to verify that their instrument meets operational requirements. This is designated by global pharmacopeias, which prescribe how to test the operational range of the instrument. These tests are conveniently automated in the Cary UV Workstation software—allowing laboratories to readily verify if their instrument is multipharmacopeia (USP, EP, JP) compliant.[7]

Conclusion

The Agilent Cary 3500 Flexible UV-Vis spectrophotometer with variable-path-length cell holder demonstrates exceptional performance in the measurement of ethanol impurities according to global pharmacopeia standards. This underscores the system's suitability for pharmaceutical-grade quality control. Combined with Agilent Cary UV Workstation software and a 10-year xenon flash lamp replacement warranty, this system delivers a user-friendly interface and global pharmacopeia-compliant hardware (USP, EP, JP), providing long-term stability and low maintenance. Optimized workflows enable precise, routine photometric quality checks to ensure products are in compliance with pharmaceutical-grade standards.

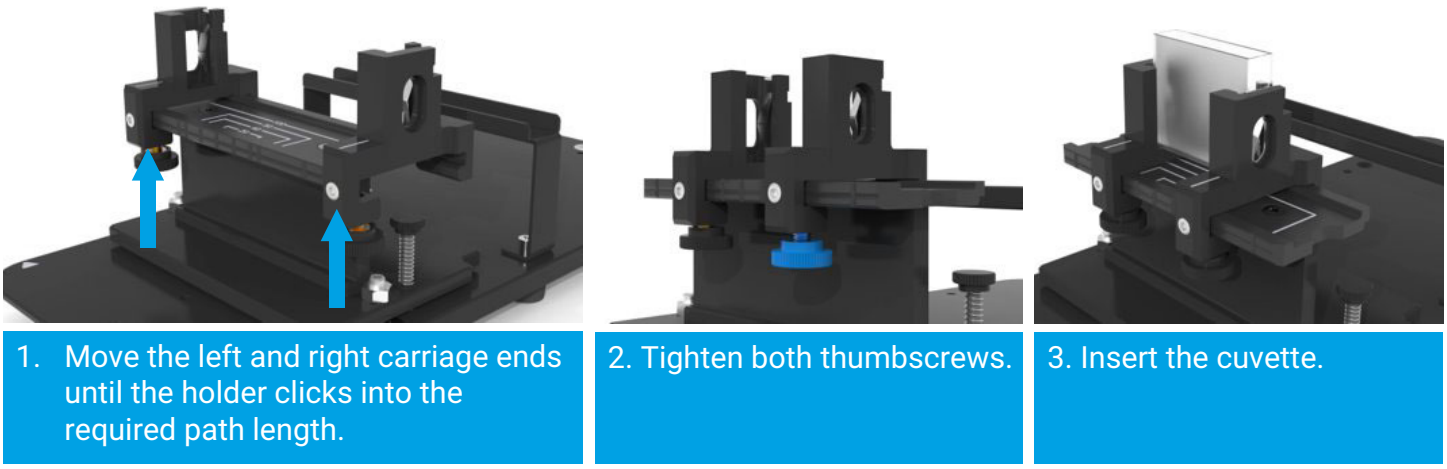

Figure 5. The Agilent Cary 3500 Flexible UV-Vis with variable-path-length cell holder. The holder features built-in notches at specific path lengths, allowing the carriage ends to click into place for quick, easy changes that can accommodate various cell types.

Figure 6. Overview of the advantages of the Agilent Cary 3500 Flexible UV-Vis spectrophotometer for quality control in the pharmaceutical industry.

References

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Further information

- Agilent Cary 3500 Flexible UV-Vis spectrophotometer
- Cary UV Workstation Software
- UV-Vis Spectroscopy Learning Tools
- UV-Vis & UV-Vis-NIR Instrument Selection Guide
- UV-Vis Spectrophotometer Applications Overview
- UV-Vis Spectroscopy FAQs

www.agilent.com/chem/cary-3500-flexible-uv-vis

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