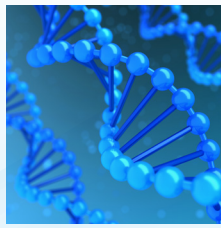
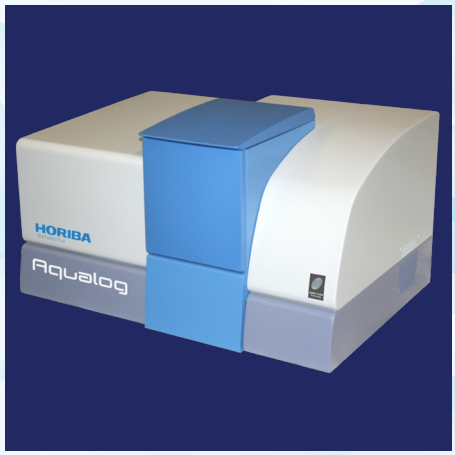
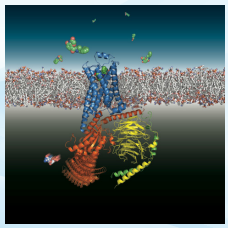
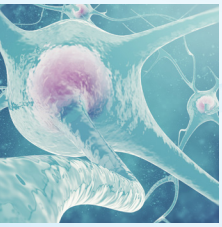
HORIBA
Scientific

Validation of the Performance of the HORIBA Aqualog® for Pharmaceutical and Industrial Analyses









A fast and inexpensive optical technique for quantitative molecular fingerprinting

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Validation of the Performance of the

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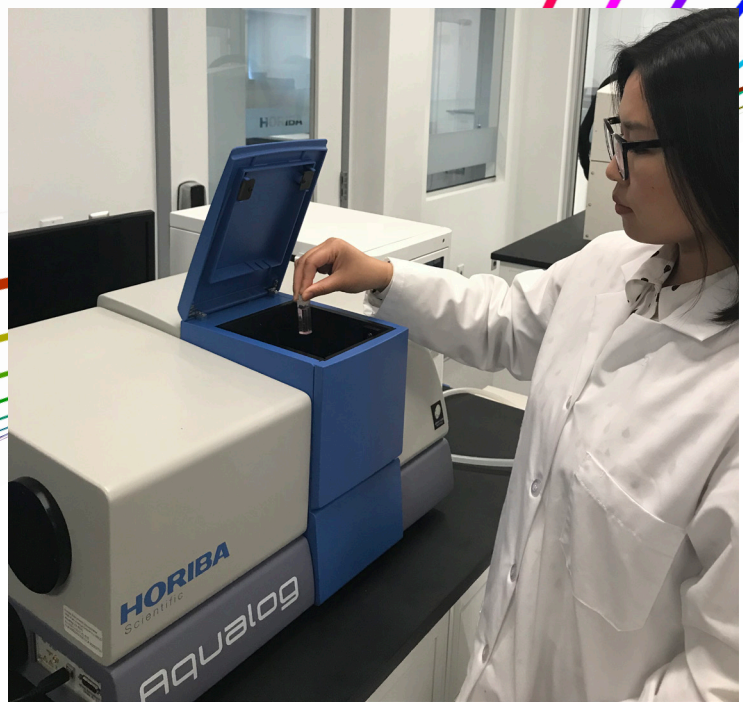
The Aqualog UV-800 is a compact optical spectrometer that is used for vaccine QC, cell media qualification and other applications in biopharma.



Fluorescence spectroscopy is a powerful technique complementing UV-VIS absorbance as a ubiquitous technique in the many hundreds of thousands of laboratories around the world. With the introduction of the patented Aqualog A-TEEM™ (Absorbance-Transmission fluorescence Excitation and Emission Matrix) method (ref1) HORIBA Scientific has created a new analytical instrument that provides quantitative molecular fingerprints with real-time Inner Filter Effect correction (IFE). This is accomplished in the patented configuration of the Aqualog CCD spectrometer which quickly acquires 3D fluorescence Excitation Emission Matrices (EEMs) simultaneously with the UV-VIS absorbance spectrum. IFE correction is important because it facilitates a wider linear concentration range for fluorescence spectral component information, which is important for component identification and quantification when employing uni- or multivariate analyses commonly applied in pharmaceutical and other life science industries. The main purpose of this document is to explain how HORIBA's Aqualog absorbance and fluorescence performance parameters are validated using certified NIST-traceable criteria to support compliance with respective United States Pharmacopoeia (USP) chapter guidelines.

Validation of HORIBA's Aqualog: An A-TEEM Spectrometer

The HORIBA Aqualog is a simultaneous fluorescence and absorbance spectrometer that enables rapid acquisition of an A-TEEM molecular fingerprint[1]. In addition to offering significantly expanded capabilities compared to either fluorescence or absorbance measurements acquired separately, the combined platform has a clear path to traceable validation, following standard established protocols for each. This includes certified validation of all major absorbance and fluorescence performance specifications consistent with the US Pharmacopoeia methods, namely, USP<857> and <853>, respectively (which are part of USP 40)[2,3] published guidelines.

HORIBA's Aqualog A-TEEM Calibration/Validation Toolbox

The Aqualog software is fully equipped to facilitate both calibration and validation of all instrumental absorbance and fluorescence performance functions, including the following tests:

- Photometric accuracy
- Absorbance and Fluorescence Excitation Wavelength Accuracy
- Stray Light
- Emission Wavelength Accuracy
- Emission Spectral Shape Accuracy
- Water Raman Band Accuracy
- Water Raman Band Signal to Noise (SNR)
- Water Raman Band Area Scattering Units (RSU)
- Quinine Sulfate Units (QSU)

The Aqualog toolbox is modular and intuitive, and along with HORIBA IQ/OQ documentation, guides users through the validation process. Individual Pass/Fail Tests can easily be selected individually to minimize test redundancy and facilitate more routine testing requirements.

Measuring Absorbance and Excitation Wavelength Accuracy and Excitation Spectral Correction

The Aqualog absorbance and excitation wavelength accuracy is certified using the Holmium oxide sample in the USP (RMLKI) kit from Starna Inc., which is specifically calibrated for 5 nm optical bandpass. The excitation spectral shape correction over the complete spectral range (200-800 nm) is implemented using a NIST-traceable calibrated photodiode (International Light Technologies Inc., Peabody, MA USA) as described previously[4].

Measuring Water Raman Sensitivity and Wavelength Calibration

Water Raman Sensitivity and emission wavelength calibration are both determined by measuring with a sealed Certified Reference Material (CRM) sample of pure water. The Raman Peak is evaluated by fixing the excitation at 350 nm and measuring the peak at 397 nm, the area under the Raman band from 365-450 nm, the baseline intensity at 450 nm and Root Mean Square (RMS) noise for 5 nm around 450 nm. The Aqualog uses a fixed 5 nm optical bandpass, 0.5 nm emission wavelength increments and a 30 s dark-count integration time.

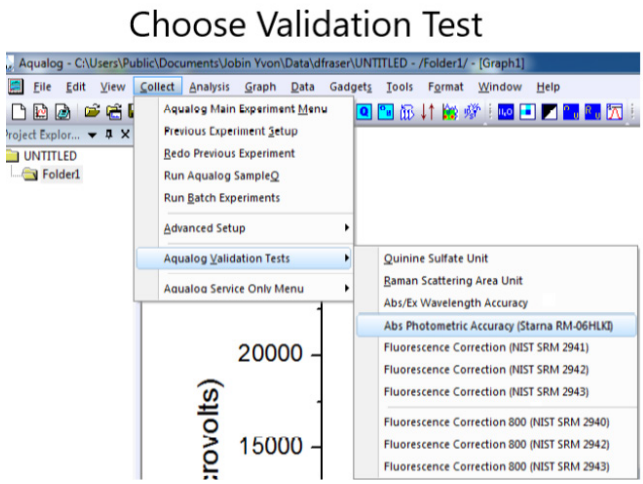
The Water Raman SNR is evaluated in RMS units as previously described for purposes of instrument throughput specification[4]. The precision and accuracy of the Raman peak (397±1 nm) is also used to specify the instrument's excitation and emission wavelength accuracy on a routine basis.

The Raman band area is used as an external standard for normalizing fluorescence throughput using Raman Scattering Units (RSU) to compare instruments' signals. RSU values are evaluated relative to the integration time and CCD camera binning and gain settings for purposes of dynamic signal adjustment. RSU normalization is primarily used to account for daily and day-to-day signal changes as they are influenced by the lifetime and intensity of the excitation light source. RSU normalization can also be used to adjust and optimize signals to account for concentration and avoid detector saturation for strong samples, or for increasing integration time for weak samples. It is noted that the Aqualog uses a CCD for fluorescence detection so units of intensity are not directly equivalent to analog or photon counting mode signals from a conventional scanning photomultiplier instrument.

For convenience, the water CRMs are sold in sealed cuvettes in order to help prevent microbiological growth and accumulation of airborne particles or pollutants inside the cuvette over time. Water Raman CRMs sourced from Starna Inc. and resold by HORIBA Instruments Inc. are provided with a serial number and recertification/expiration dates which are administered by Starna Inc.

Measuring Photometric Accuracy and Linearity

Photometric accuracy of the Aqualog is important because 1) it is both an absorbance and fluorescence spectrometer, and 2) because it is designed to use the absorbance information for IFE correction of the fluorescence excitation and emission spectra[4]. We certify both the photometric accuracy and linearity using the Potassium Dichromate samples (20 mg/L, 60 mg/L and 100 mg/L) in Starna's USP kit calibrated for 5 nm optical bandpass.



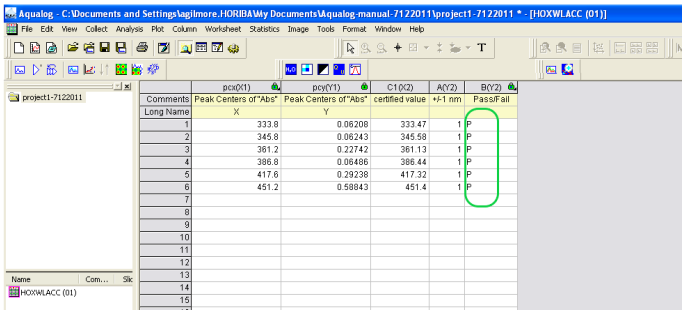
Measuring Stray Light

The Aqualog uses a double-subtractive excitation monochromator incorporating blazed-holographic gratings to provide excellent stray light rejection. Thus, stray light from the powerful excitation light source (150 W xenon lamp) in the Aqualog is strongly attenuated, compared to a conventional scanning spectrometer using a single grating excitation monochromator. We evaluate stray light according to USP 8573 using the 5 mm and 10 mm pathlength Potassium Iodide, Sodium Nitrite and Acetone samples in Starna's USP kit, calibrated for 5 nm optical bandpass.

Spectral Correction of Fluorescence Emission

The fluorescence spectral emission for the Aqualog is both corrected and certified using Standard Reference Materials sourced directly from NIST, namely SRMs 2940, 2942 and 2943[4]. Fluorescence spectral correction, wavelength accuracy and external intensity normalization (Quinine Sulfate Units) can also be verified using the NIST-traceable CRM sourced from Starna Inc. (RMQS-00). QSU normalization, like RSU, is fully supported in the Aqualog software to account for the integration time and CCD binning and gain parameters settings.

Acquire Data and View Results of Validation Test



Installation Operation Qualification (IQ/OQ)

The Aqualog is fully supported for pharmaceutical and industrial installations with a comprehensive IQ/OQ document and protocol. The IQ/OQ takes into consideration all instrument performance certifications and software functionalities associated with 21 CFR Chapter 11 compliance, as required for current Good Manufacturing Practices (cGMP).

References

1. AM Gilmore, X Tong (2018) SYSTEM AND METHOD FOR FLUORESCENCE AND ABSORBANCE ANALYSIS. US Patent 10184892
2. US Pharmacopoeia, USP<853> (Part of USP 40).
3. US Pharmacopoeia, USP<857> (Part of USP 40)
4. AM Gilmore (2014) How to Collect National Institute of Standards and Technology (NIST) Traceable Fluorescence Excitation and Emission Spectra. Methods Mol Biol. 1076, 3-27.
5. DeRose PC, Early EA, Kramer GW (2007) Qualification of a Fluorescence Spectrometer for Measuring True Fluorescence Spectra. Rev Sci Instrum 78:033107–033112.

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