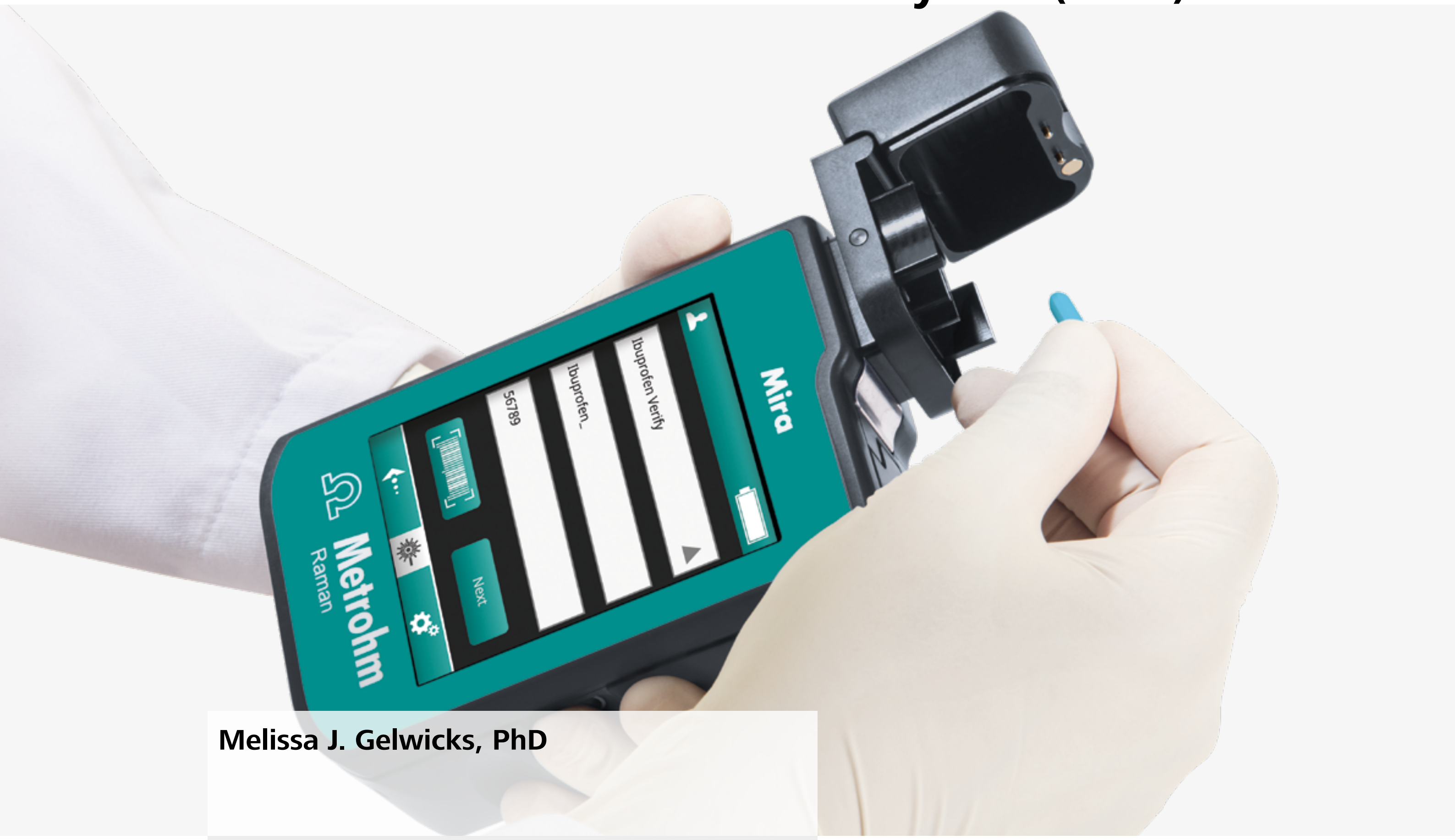
Instrument Calibration, System Verification, and Performance Validation for Metrohm Instant Raman Analyzers (Mira)



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Analytical Instrument Qualification (AIQ) according to the United States Pharmacopeia (USP) ensures that instruments perform as intended *and* users may have confidence in data quality. As the Pharma industry adopts handheld Raman instruments for incoming materials identification and verification, producers of such systems must provide suitable calibration and validation routines. Upon completion of these tests, end users are assured that all measurements are in accordance with agreed standards- at Metrohm Raman, we have sophisticated AIQ routines in place to confirm the quality of your results.

Metrohm International Headquarters; Herisau, Switzerland

Metrohm Raman

This document describes Instrument Calibration, System Verification, and Performance Validation in terms of both Raman theory and government norms & standards and summarizes these tests in the System Suitability Test (SST) for Mira P.

Figure 1: Mira P Raman Analyzer

Background

Raman spectroscopy results from inelastic scattering of incident laser photons on a material, producing Raman scattered light that is shifted in energy from that of incident photons. This is expressed as a spectrum when the Raman signal (Intensity, y-axis) is plotted against shifted energies, expressed as Wavenumbers (cm^{-1}) on the x-axis. It is common to experience widely varying Raman spectra due the efficiency of optical transmission and detector response, which can be resolved by calibration and standardization. USP, in collaboration with National Institute of Standards and Technology (NIST), American Society for Testing and Materials (ASTM), European Pharmacopoeia (EP), and research groups, defines routines and Standard Reference Materials (SRM) for standardization of Raman spectrometers.

Instrument Calibration

Calibration of Raman instruments «corrects instrument-induced spectral artifacts», as required by USP <1858> and <858>. This confirms measurement accuracy and repeatability, and, on a larger scale, it results in universal Raman libraries, spectra, and instruments. There are three components to instrument calibration:

- Wavenumber (cm^{-1}) calibration or correction of absolute peak positions on the **x-axis** of a Raman spectrum
- Intensity calibration or relative intensity response expressed on the **y-axis**
- Laser calibration

X-Axis Calibration (TA Standard)

The integrity of peak positions on the x-axis of Raman spectra is established by Raman Shift Standards for Spectrometer Calibration, ASTM E1840-96. Accurate wavenumber shifts with standard deviations less than 1 cm^{-1} have been carefully established for a small handful of chemicals, chosen to represent a full spectral range for Raman spectrometers (350–2 300 cm^{-1} for handheld instruments.) Metrohm Raman uses a 1:1 v/v mixture of Toluene:Acetonitrile for x-axis calibration, with an acceptance criteria of ±2 cm^{-1}, exceeding criteria for handheld Raman systems (±2.5 established by ASTM E1840).

Raman spectroscopy is a fingerprint technique for identification and verification of materials. Each material has a unique Raman spectra, with peaks representing the unique chemical bonds in a substance. At the most fundamental level, x-axis calibration assures accurate material identification by mapping standard wavenumber peaks on the Raman shift axis to published values.


Certificate of Analysis

ISO GUIDE 34 ANAB Cert# AR-1470

ISO/IEC 17025 ANAB Cert# AT-1467

ACETONITRILE/TOLUENE (1:1)

CERTIFIED REFERENCE MATERIAL

NOMINAL PACKAGE SIZE: 1.8mL

CATALOG #: 13000-5501-C LOT #: LRAB8828 Turquoise

CERTIFICATE VERSION: LRAB8828.1 ISSUE DATE: 14 June 2018

CRM EXPIRATION: 31 December 2022 (Proper Storage and Handling Required).

RECEIPT DATE: ______

Note: this space is provided for convenience only and its use is not required.

STORAGE: Store in a Room Temperature, do not open vial.

CHEMICAL FORMULA: CH_3CN; C_7H_8 MW: N/A

PHYSICAL DESCRIPTION: Clear liquid in clear vial CAS #: N/A

HAZARDS: Read Safety Data Sheet before using. All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel.

INSTRUCTIONS FOR USE: This material is intended for Laboratory Use only. Not for drug, household or other uses.

Wavenumber Theoretical:	Wavenumber Certified Value (±U_{crm}) vs. PHR1551, LRAB0293 PHR1311, LRAB1079	Assigned Content of: PHR1551, LRAB0293 PHR1311, LRAB1079
378.5 cm^{-1}	**378.6 ± 0.8 cm^{-1}**	378.5 cm^{-1}
521.7 cm^{-1}	**521.7 ± 0.5 cm^{-1}**	521.7 cm^{-1}
786.5 cm^{-1}	**786.5 ± 0.5 cm^{-1}**	786.5 cm^{-1}
1003.6 cm^{-1}	**1003.7 ± 0.6 cm^{-1}**	1003.6 cm^{-1}
1211.4 cm^{-1}	**1211.4 ± 0.4 cm^{-1}**	1211.4 cm^{-1}
1605.1 cm^{-1}	**1605.1 ± 0.5 cm^{-1}**	1605.1 cm^{-1}
2253.7 cm^{-1}	**2253.8 ± 0.4 cm^{-1}**	2253.7 cm^{-1}

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Figure 2: Certification for TA Standard

Y-Axis Calibration

Y-axis calibration is a less intuitive, but no less important, adjustment. Identification of materials with Raman spectroscopy relies on comparison of measured spectra within established spectral libraries. In addition to absolute peak position, peak height and shape affect accurate library comparison and must be standardized.

Standardization of Raman spectrometers is accomplished through intensity correction, as required by USP <1858> Method B and traceable to NIST SRM 2241. Metrohm Raman uses a «NIST traceable» method for y-axis correction, where a traceable correction to an established standard is applied to all spectra collected by a system, setting an internal standard to which all future measurements are corrected.

To be specific: NIST SRM 2241 is a chromium-doped green glass standard specific for Raman spectroscopy with an excitation wavelength of 785 nm. This is the established «true spectrum» of the standard to which all other measured spectra are compared. Essentially, the ratio between the true and measured spectra is measured and applied as a correction to all future acquisitions. After this NIST SRM correction is applied by spectral processing algorithms on each system, it is verified with the same TA calibration standard used at Metrohm Raman for x-axis calibration.

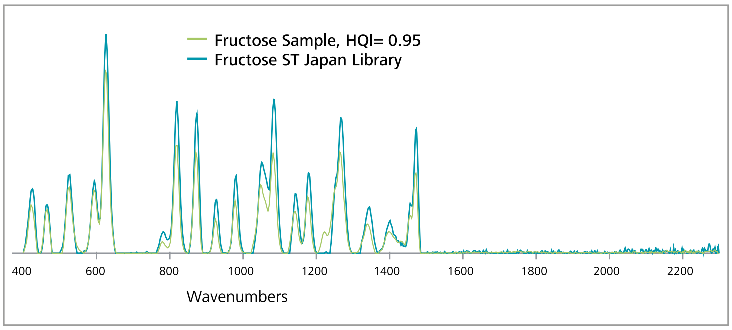


Figure 3: Raman Spectral Library Matching

Laser Calibration

The use of specific NIST calibration standards and ASTM methods for Raman instruments operating at a *wavelength of 785 nm means that laser calibration for each instrument is unnecessary.*

System Verification

Verification of calibration routines and general system fitness ensures that an instrument is performing correctly and according to claims from the manufacturer. The system verification portion of Metrohm Raman's SST report includes verification of the health and alignment of individual components, as described in USP <1858>. Voltage inputs, laser and raster health, acceptable loads, signal/noise, and the display are all assessed and reported as part of the verification routine.

Figure 4: Mira P with Calibrate/Verify Accessory

Performance Validation

Calibration and verification are sufficient for qualification of most Raman instruments. Mira P from Metrohm Raman is a handheld system *specifically designed for use by regulated industries* such as pharmaceutical, cosmetic and food producers. A new accessory for this advanced system, the Calibrate Verify Attachment (CVA) adds another level of confidence to AIQ, as required by USP <858>. USP <858> defines AIQ very specifically in terms of acceptable procedures, accuracy and precision, limits, and range for validation and verification of Raman spectrometers. The validation routine adopted by Metrohm Raman:

- Confirms system performance over time
- Demonstrates that system calibration and verification is performed with suitable accuracy, sensitivity, and precision
- Confirms wavenumber calibration
- Adheres to standards required by EP 2.2.48 and 21 CFR Part 211

Calibrate/Validate Accessory (CVA)

CVA includes the aforementioned TA standard, in addition to a second accepted standard for AIQ of Raman instruments: polystyrene.

Polystyrene is a stable, nontoxic organic substance approved (NIST SRN 706a) for validation of Raman instruments according to USP and EP 2.2.48. Wavenumber calibration reconfirms the health of a system. This is a powerful test; user adherence to 21 CFR Part 211 is implicit upon successful AIQ with polystyrene.

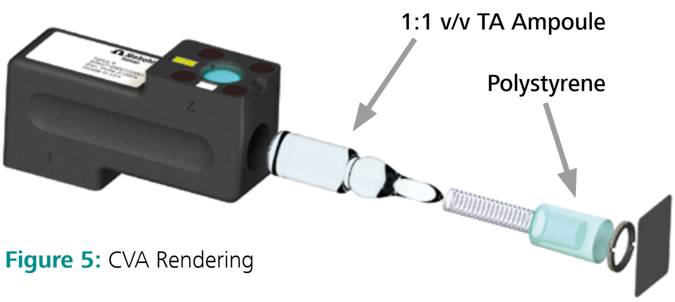


Figure 5: CVA Rendering

SST Report

Each time a system is subjected to calibration, the resulting SST can be archived as part of a traceable audit trail. A sample SST with annotation follows:

- The **Test Created** field is a record of the actual time and date of calibration.
- **Overall Status** is a simple Pass/Fail indication of the health of the system.
- **Package Version** indicates the current firmware on the device.
- The **Calibration Standard** has a lifetime of 2 years.
- **Verification** results summarize specific values reported in subsequent sections.

System Suitability Report

Metadata

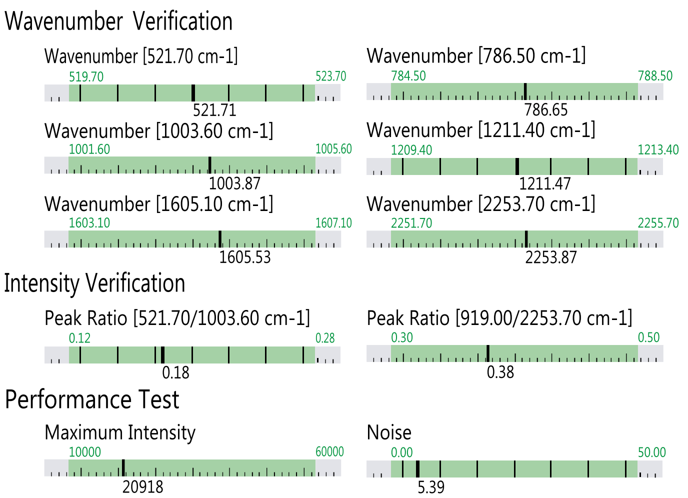
Test Created	8/27/2018 5:31:55 PM -06:00
Overall Status	Pass

Instrument

Type	Mira P
Serial Number	192600200301042
Package Version	9.0.0.27
Calibration Standard Serial Number	6060710400201043
Calibration Standard Expiration Date	12/31/2020

Verification

Wavenumber Verification	Pass
Intensity Verification	Pass
Performance Test	Pass
Voltage Verification	Pass
Performance Verification	Pass



Calibration

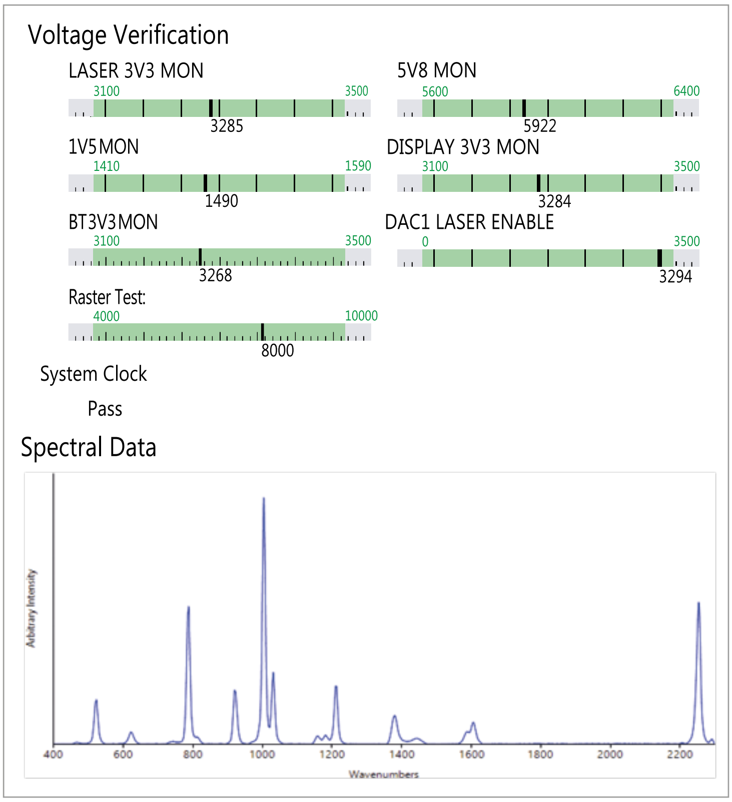
Wavenumber verification confirms absolute wavenumber peak position in the combined spectra of a 50:50 mixture of Toluene: Acetonitrile. TA is an ASTM standard for calibration of Raman shift frequencies and represents peaks across the whole Raman spectral range of 400–2 300 cm^{-1}. Generally, this is a test of component alignment; specifically it ensures integrity of peak positions.

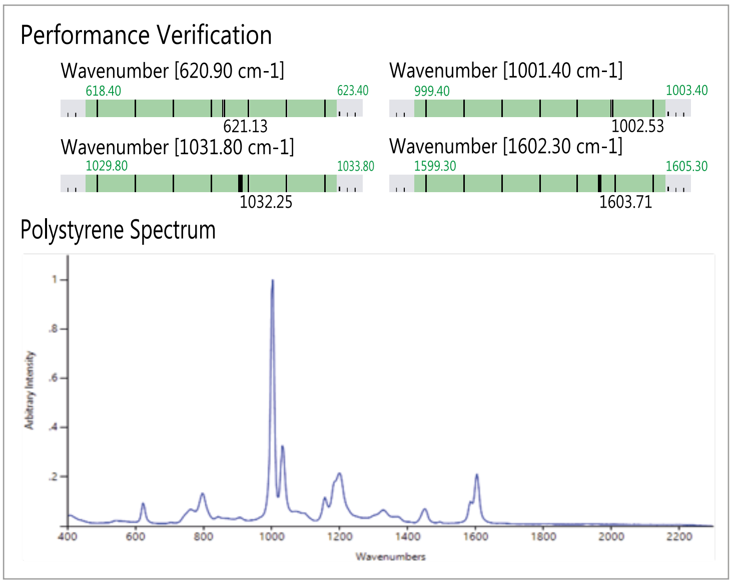
Intensity verification reflects the health of y-axis calibration and correction. Y-axis calibration is a test of the efficiency of every optical component in a system including lenses, filters, and detectors. Intensity verification also evaluates correction algorithms applied by the manufacturer. The peak ratios for toluene (left) and acetonitrile (right) reported below indicate that appropriate correction algorithms have been applied.

Verification

Performance Tests assess the health of the laser and inherent instrument noise. Appropriate values for Maximum Intensity, based on the height of the 1003.6 cm^{-1} toluene peak, indicate that the instrument is passing and detecting light correctly. Noise measurements are taken from a non-Raman-active portion of the sensor to reflect the actual spectral noise of the CCD. These tests determine whether instrument signal and noise performance are within acceptable parameters set by the manufacturer as suggested by USP 40-NF 35 <1058>.

Validation





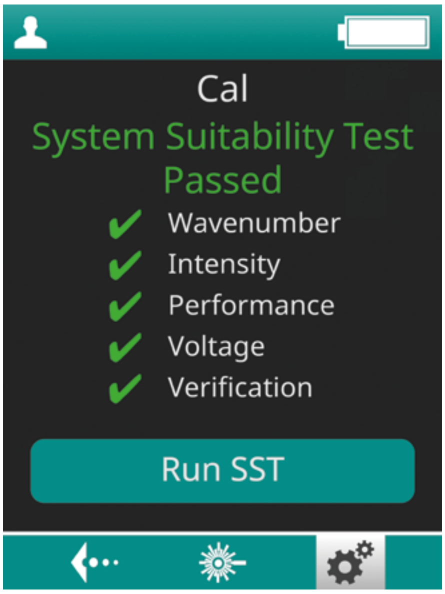


Figure 6: SST Passed Screenshot from Mira P

Voltage Verification routines assess the health of a system, from an electrical perspective. Information about all electrical subsystems can be found here.

The **Raster Test** is unique to Metrohm Raman spectrometers, which are equipped with Orbital Raster Scan (ORS) technology. This is the actual RPM of the raster at the time of testing. If the raster test fails, the laser interlock is tripped and the laser will not function.

The **System Clock** indicates that the system is in agreement with real time (UTC, confirmed each time the instrument is connected to a computer), ensuring data integrity.

The **TA Spectrum** is the actual spectrum collected by the instrument during calibration. It provides immediate visual confirmation of the quality of collected data and can be used to confirm many of the measurements reported here.

Performance Verification is an additional layer of assurance that a system is functioning properly. This calibration confirms the accuracy of Raman peak positions within acceptable range in accordance with EP 2.2.48. Again, the actual **Polystyrene Spectrum** that was collected during Instrument Calibration is included to provide visual confirmation of reported numerical results.

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