Handheld Raman Spectrometer BRAVO

Statement of Compliance to Pharmaceutical Guidelines & Regulations

United States Pharmacopoeia (USP), chapter <858> and <1858>

European Pharmacopoeia (Ph. Eur.), chapter 2.2.48

Japanese Pharmacopoeia (JP), chapter 2.26

Chinese Pharmacopoeia (Ch. P. 0421)

21 CFR Part 11, Data Integrity, (c)GMPs, Eudralex Annex 11 & 15, ICH Q7, ...

The handheld Raman spectrometer BRAVO is a dedicated analyzer for raw materials verification and is developed to meet highest standards of the Pharmaceutical industry.

The following document outlines in detail how the BRAVO spectrometer and software do meet the manifold guidelines & regulations of the of the Pharmaceutical industry.

Part 1 focusses on performance requirements set by Pharmacopoeias and **Part 2** on software solutions to meet the standards of leading regulations (GMPs, 21 CFR Part 11, Data Integrity, ...).

PART 1: Pharmacopoeias

Pharmacopoeias define directions for the quality control of medicines and describe applicable analysis methods and their respective minimum performance requirements. The United States (USP) and European (Ph. Eur.) Pharmacopoeias are the globally leading editions, and their requirements are discussed in the following.

1.1. USP (USP43-NF38 2S)

<858> and <1858> are the USP chapters specific to Raman spectroscopy, which had been introduced November 1st, 2020, replacing the former well-established chapter <1120>.

1.1.1 USP <1858> Raman Spectroscopy - Theory and Practice

This chapter provides a general description of Raman spectroscopy as technique and mentions possible applications in the Pharmaceutical industry. There are a few resulting hardware requirements, for example in the **Calibration** section.

USP<1858>: *"Raman instrument calibration involves three components: primary wavelength (x-axis), laser wavelength, and intensity (y-axis)"*

BRAVO uses an internal atomic emission lamp (neon) to perform a primary x-axis calibration. Subsequently, the Raman shift or respective laser wavelength calibration is performed using several certified Raman shift standards according to ASTM E1840 - 96(2014) (acetaminophen, benzonitrile and sulfur). The materials had been carefully selected to cover the full spectral range of 300 to 3200 cm^{-1}.

Intensity calibration (y-axis) is performed using a NIST SRM 2241 relative intensity correction standard for Raman spectroscopy. This approach is referred to as method B in USP <1858>.

USP <1858>: *"The validity of this calibration approach can be verified after laser wavelength calibration by using a suitable Raman shift standard."*

"The Raman shift wavelength(s) for a given material must therefore be confirmed to ensure that the wavelength scale is accurate for both FT-Raman and dispersive Raman instruments. A reference Raman shift standard material such as those outlined in American Society for Testing and Materials (ASTM) E1840 or other suitably verified materials, where the observed peak position(s) have been verified, can be used for this purpose."

"Detailed functional validation using external reference standards is recommended to demonstrate instrumental suitability of laboratory instruments, even for instruments that possess an internal calibration approach."

Bruker offers several certified Raman shift standards according to ASTM E1840 - 96(2014) to verify the Raman shift accuracy (acetaminophen, benzonitrile, cyclohexane, naphthalene, polystyrene) in scope of automated, qualified test routines of the OPUS Validation Program (OVP). Detailed performance requirements are set in USP <858> and are discussed in the following.

USP <1858>: *"In all instruments suitable for analytical Raman measurements, the vendor offers a procedure for x-axis calibration that can be performed by the user."*

"Unless the instrument is of a continuous-calibration type, the primary wavelength (x-axis) calibration should be performed, as per vendor procedures, just before measuring the laser wavelength. For external calibration, the Raman shift standard should be placed at the sample location and measured using appropriate acquisition parameters."

The functionality for x- and y-axis calibration are implemented in the BRAVO software and follow a defined procedure. The calibration is initially performed at factory and is of continuous type. Thus, specific calibration intervals or frequent corrections are not required. Of course, most extensive verification procedures allow the user to confirm a valid calibration (OVP) at any time. Additionally, using the inbuilt neon lamp prior every measurement a verification of the primary x-axis is performed and information is stored in the parameter section of each OPUS spectrum file.

Furthermore, USP <1858> discusses **sample-based factors which do affect the measurement performance**. Here, fluorescence is the most important aspect identified by USP <1858>.

USP <1858>: *"The most important sample-based factor that deleteriously affects quantitative Raman spectroscopy is fluorescence."*

"Fluorescence is the primary variable that can affect the suitability of a procedure. The presence of fluorescent impurities in samples can be quite variable and can have little effect on the acceptability of a material."

This holds certainly as well for qualitative measurements, especially as fluorescence levels can easily vary from sample to sample. With Sequentially Shifted Excitation (SSE™), BRAVO features a unique, patented active fluorescence mitigation suppressing the impact of fluorescence while maintaining high sensitivity (i.e. in avoiding the use of NIR excitation such as 1064 nm).

USP <1858>: *"The effect of the laser on the sample must be determined. Visual inspection of the sample and qualitative inspection of the Raman spectrum will confirm that the sample is not being altered (other than by photobleaching)."*

This argument is frequently overlooked, as Raman spectroscopy typically is denoted as a non-destructive analysis technique. But high-power lasers are used for excitation resulting in high power densities at the focus position, respectively at the sample. As a laser class 1 product BRAVO avoids higher laser output powers and additionally a non-circular beam shape reduces maximum available power densities, minimizing the risk for sample alteration.

1.1.2 USP <858> Raman Spectroscopy

In contrast to USP <1858>, chapter <858> defines explicit performance criteria, which Raman spectrometers in the Pharmaceutical industry need to comply to.

USP<858>: *"Qualification of Raman spectrometers is divided into three components: 1) installation qualification (IQ); 2) operational qualification (OQ); and 3) performance qualification (PQ)."*

With the Bruker System Validation Manual, a detailed documentation is offered assisting the complete qualification process. Especially, the validation manual offers description and test logs forms for installation (IQ), operational (OQ) and performance qualification (PQ). Respective performance requirements can be verified in scope of the OQ and PQ test protocols of the OVP.

For **Operational Qualification** (OQ), USP <858> defines several general acceptance criteria.

Wavelength Accuracy

USP <858>: *"The instrument wavelength accuracy can be determined by using a Raman-shift standard or other suitably high-purity material (e.g., acetaminophen, cyclohexane, or polystyrene). Selection of a standard with bands present across the full Raman spectral range is recommended so that instrument wavelength accuracy can be evaluated at multiple locations within the spectrum."*

"For qualitative requirements, the maximum allowable acceptance criteria for all wavelengths contained in Table 1 is ±3.0 cm^{-1}."

BRAVO's automated OQ and PQ test routines enable the user to verify the wavelength accuracy across the full spectral range, using certified reference materials according to ASTM E1840 - 96(2014), explicitly acetaminophen, benzonitrile, cyclohexane, naphthalene, and polystyrene. The acceptance criteria are set according to Ph. Eur. 2.2.48 for handheld spectrometers, defining more narrow accuracy limits than the general ± 3.0 cm^{-1} acceptance threshold as introduced by USP. Notably, the BRAVO is as well capable to comply to the requirements set by USP for quantitative analysis.

Photometric Precision

USP <858>: *"Laser variation in terms of the total emitted photons occurring between two measurements can give rise to changes in the photometric precision of the instrument."*

"...; a maximum tolerance of 10% from reference measurements made from the reference material is applied."

BRAVO's Photometric Precision test as part of the OQ and PQ test routines verifies the absolute Raman intensities being within the set 10 % acceptance limit for subsequent measurements. As this performance requirement was newly introduced November 1st, 2020 Bruker provides an official released test script to cover this new requirement for already installed systems.

With the introduction of USP <858> **Performance Qualification** (PQ) does not anymore ask for explicit acceptance criteria such as USP <1120> did. Instead, it is suggested that an application specific system suitability test (SST), is appropriate to verify the performance in routine. This certainly holds for equipment installed in a process environment,

where it might not anymore possible to verify all parameters requested to be challenged in scope of Operational Qualification (OQ). For a handheld Raman spectrometer, it is well possible, without explicit efforts, to monitor these performance parameters as indirectly suggested by USP <858>:

USP 858: *"...when the instrument has been set up for a specific measurement, it might no longer be possible or desirable to measure the wavelength and photometric (signal) qualification reference standards used in the OQ. Provided that instrument OQ has shown that the system is fit for use, a single external performance verification standard may be used on a continuing basis (e.g., daily or before use)."*

BRAVO's PQ test routines applied for daily performance qualification consider all performance requirements as set forth by USP for Raman spectroscopy. All criteria can be verified using a single NIST traceable polystyrene reference material, ensuring a time efficient daily qualification process.

1.2. Ph. Eur. 2.2.48 Raman Spectroscopy (European Pharmacopoeia 10.7)

As well Ph. Eur. 2.2.48 sets requirements for the control of the instrument performance. Other than USP <858>, Ph. Eur. 2.2.48 does not distinguish test criteria for operational and performance qualification and requires performance parameters, which are outlined in the following, to be verified at regular intervals. Therefore, like for the USP test criteria all following criteria defined by Ph. Eur. 2.2.48 are monitored in scope of the routine performance qualification tests of the OVP.

Wavenumber Scale

Ph. Eur. 2.2.48: *"Verify the wavenumber scale for Raman shift using a suitable standard that has characteristic maxima at the wavenumbers under investigation, for example an organic substance such as polystyrene, paracetamol or cyclohexane (see Table 2.2.48-1)."*

"A minimum of 3 wavenumbers covering the working range of the instrument intended for measurements should be selected."

BRAVO's automated OQ and PQ test routines enable the user to verify the wavelength accuracy across the full spectral range, using certified reference materials according to ASTM E1840 - 96(2014), explicitly acetaminophen, benzonitrile, cyclohexane, naphthalene, and polystyrene. The verification of the wavenumber scale is performed at minimum of 3 Raman bands for each substance. The acceptance criteria are set according to Table 2.2.48-1 for handheld spectrometers. Notably, the BRAVO is as well capable to comply to the requirements set by Ph. Eur. for benchtop instrumentation.

Response-Intensity Scale

Ph. Eur. 2.2.48: *"Appropriate acceptance criteria will vary with the application. A maximum variation of ± 10 per cent in band intensities compared to the previous instrument qualification is achievable. Response calibration may involve the use of white-light standards or luminescent glass (e.g. NIST SRM 2241)."*

Response-intensity calibration (y-axis) is performed using a NIST SRM 2241 relative intensity correction standard for Raman spectroscopy. BRAVO's automated OQ and PQ test routines enable the user to verify the response-intensity scale applying the mentioned 10 % acceptance threshold in relative band intensities (Photometric Consistency test). The evaluation is performed with respect to a reference measurement taken at instrument calibration/qualification.

Note, for OPUS versions up to 8.5, this test for y-axis calibration is called Photometric Precision test, as it was called in the former valid USP chapter <1120>. With introduction of USP <885>, the meaning of the Photometric Precision test was altered. Thus, in latest OPUS software versions, a new Photometric Precision test was implemented in the OVP program and the test for y-axis calibration was renamed to Photometric Consistency test.

Spectral Resolution

Ph. Eur. 2.2.48: *"For identity tests, unless otherwise prescribed in a monograph, the spectral resolution must be less than or equal to 15 cm^{-1} (measured in the wavenumber range between 1000 cm^{-1} and 1100 cm^{-1})."*

The spectral resolution of the BRAVO spectrometer is verified by the OVP program to be better than or equal to 9.8 cm^{-1}
with an allowed tolearnce of +10 % for the 1085 cm^{-1} Raman band of calcium carbonate. The calculations for determining the spectral resolution are in line to the mentioned formular, which corresponds to the criteria of the ASTM standard guide for testing the resoluition of a Raman spectrometer E2529-06(2014).

Note, this performance criteria was only introduced with Ph. Eur. 10.7, but is already scope of the OVP performance testing for already installed BRAVO spectrometers.

In line to USP, Ph. Eur. 2.2.48 mentions fluorescence as one of the major difficulties faced with Raman spectroscopy.

Ph. Eur. 2.2.48: *"A major difficulty of Raman spectroscopy is that the material material (or its impurities) under examination may exhibit fluorescence, which can can mask the Raman signal. Fluorescence may be avoided by choosing a longer excitation wavelength, for example in the near-infrared region but at the cost of lower Raman scattering efficiency and thus longer analysis times. In addition, special hardware or software can contribute to limit the impact of fluorescence."*

With Sequentially Shifted Excitation (SSE™) BRAVO features a unique, patented active fluorescence mitigation suppressing the impact of fluorescence while maintaining high sensitivity (i.e. in avoiding the use of NIR excitation such as 1064 nm).

1.3. Other Pharmacopoeias

With Raman spectroscopy gaining popularity in Pharmaceutical raw materials control, Raman spectroscopy was introduced to further Pharmacopoeias such as the Japanese (JP 2.26) and Chinese (ChP 0421) editions. Because USP and Ph. Eur. represent a kind of global standard these local editions are with respect to Raman spectroscopy harmonized in all relevant aspects and automatically covered by the measures taken to comply to USP and Ph. Eur.

1.4. Overview of Performance Test Requirements

In the following explicit test criteria as defined by USP <858> and Ph. Eur. 2.2.48 are summarized in a tabular form.

Table 1 gives an overview on required tests according to current USP and Ph. Eur. chapters on Raman spectroscopy, and **table 2** and **table 3** outline the specific performance requirements. **Table 4** lists additional performance tests which are included on part of Bruker for a most comprehensive monitoring of the instrument performance.

OVP Performance Test	Description	Ph.Eur. 2.2.48	USP <858>
Wavenumber Accuracy	Test for x-axis calibration	x	x
Photometric Consistency	Test for y-axis calibration	x	-
Photometric Precision	Test for y-axis precision	-	x
Spectral Resolution	Test for spectral resolution	x	-

Table 1: Explicit performance test requirements of USP <858> and Ph. Eur. 2.2.48 considered by the OPUS Validation Program (OVP). A x denotes a test requirement to be applicable.

Reference Material	Band Position [cm^{-1}] according to ASTM E1840-96(2014)	Allowed Tolerances			BRAVO OVP (OQ/PQ) Test Criteria
		Ph. Eur. (benchtop) USP (quantitative) [cm^{-1}]	**Ph. Eur. (handheld) [cm^{-1}]**	**USP (qualitative) [cm^{-1}]**	
Polystyrene	620.9	± 1.5	± 2.5	± 3.0	± 2.5
	1001.4	± 1.5	± 2.0	± 3.0	± 2.0
	1031.8	± 1.5	± 2.0	± 3.0	not tested*
	1602.3	± 1.5	± 3.0	± 3.0	± 3.0
	2852.4	-	-	-	± 3.0
	3054.3	± 3.0	NA	± 3.0	not tested*
Paracetamol	390.9	-	-	-	± 2.5
	797.2	± 1.5	± 2.5	± 3.0	not tested*
	857.9	± 1.5	± 2.0	± 3.0	± 2.0
	1168.5	± 1.5	± 2.0	± 3.0	± 2.0
	1236.8	± 1.5	± 2.0	± 3.0	not tested*
	1323.9	± 1.5	± 2.5	± 3.0	not tested*
	1648.4	± 1.5	± 3.0	± 3.0	not tested*
	2931.1	± 2.0	NA	± 3.0	± 3.0

* USP and Ph. Eur. do not require to test all individual Raman bands. Raman bands had been selected for an uniform test coverage across the complete spectral range.

Reference Material	Band Position [cm^{-1}] according to ASTM E1840-96(2014)	Allowed Tolerances			BRAVO OVP (OQ/PQ) Test Criteria
		Ph. Eur. (benchtop) USP (quantitative) [cm^{-1}]	**Ph. Eur. (handheld) [cm^{-1}]**	**USP (qualitative) [cm^{-1}]**	
Cyclohexane	801.3	± 1.5	± 2.5	± 3.0	± 2.5
	1028.3	± 1.0	± 2.0	± 3.0	± 2.0
	1266.4	± 1.0	± 2.0	± 3.0	± 2.0
	1444.4	± 1.0	± 2.5	± 3.0	± 2.5
	2852.9	± 2.0	± 3.0	± 3.0	± 3.0
Benzonitrile	460.9	-	-	-	± 2.5
	1000.7	-	-	-	± 2.0
	1598.9	-	-	-	± 3.0
	2229.4	-	-	-	± 3.0
	3072.3	-	-	-	± 3.0
Naphthalene	763.8	-	-	-	± 2.5
	1382.2	-	-	-	± 2.5
	3056.4	-	-	-	± 3.0

Table 2: Wavenumber accuracy tolerances as defined by USP <858> and Ph. Eur. 2.2.48 for selected materials and band positions. Some higher wavenumber bands in the CH stretching region are marked by Ph. Eur. as NA for handheld equipment as these bands are beyond the detector range for most handheld instruments. Furthermore, additional reference materials and band positions than outlined in USP and Ph. Eur. were added to extend the test coverage for BRAVO across the whole spectral range. Additional reference materials and literature band positions are defined accoring to ASTM E1840-96(2014). This test is part of OQ and PQ test procedures.

Performance Tests	Description	Band Position [cm^{-1}]	Acceptance criteria
Photometric Consistency required by Ph. Eur. 2.2.48	Test confirms y-axis calibration in evaluating relative band intensities towards a reference measurement	Polystyrene, according to ASTM E1840-96(2014) 620.9 1001.4 1602.3	± 10 % 0 % (normalized) ± 10 %
Photometric Precision required by USP <858>	Test confirms repeatability of intensity scale of subsequent measurements	Polystyrene, according to ASTM E1840-96(2014) 1001.4	± 10 %
Spectral Resolution Test required by Ph. Eur. 2.2.48	Confirmation of specified spectral resolution. Part of OQ procedure.	Calcite, according to ASTM E2529 - 06 1085.0	+ 10 % towards specified value of 9.8 cm^{-1}

Table 3: Photometric Consistency and Precision as well as Spectral Resolution test criteria as defined by Ph. Eur. 2.2.48 and USP <858>. These tests are part of OQ and PQ test procedures.

Further Bruker Performance Tests	Description	Band Position [cm^{-1}]	Acceptance criteria
Wavelength Precision formerly required by USP <1120>	Test confirms x-axis precision in evaluating deviations in band positions towards a reference measurement. Part of OQ and PQ test procedures.	Polystyrene, according to ASTM E1840 - 96(2014) 620.9 1001.4 1031.8 1602.3	+/- 0.3 cm^{-1} standard deviation
Laser Power Test formerly required by USP <1120>	Test for absolute Raman intensity (proportional to laser power). Part of OQ and PQ test procedures.	Polystyrene, according to ASTM E1840 - 96(2014) 1603.3 3054.3	 - 50 % - 50 %
Technical Functionality Test	Software – Firmware communication and detector noise test. Part of OQ procedure.	Calcite, according to ASTM E2529 - 06 1085.0	See chapter 6 of the system validation manual

Table 4: Additional performance tests implemented in the OPUS Validation Program (OVP).

Part 2: Code of Federal Regulations, 21 CFR Part 11, (c)GMPs, Data Integrity, EudraLex Annex 11, ICH Q7, -...

At the forefront setting a global set of rules is the US Food & Drug Administration (FDA) with the Code of Federal Regulations (CFR), establishing the term of Good Manufacturing Practices (GMPs), which are today represented by manifold provisions by different regulatory bodies.

The BRAVO is designed to be operated complying to all these regulations and a detailed documentation is given in the System Validation Manual. The most important aspects for the handheld Raman spectrometer BRAVO are summarized in the following:

Access Controls

Access to the BRAVO instrument requires User ID and password authorization. The user management supports the concept of segregation of duty (**SOD**), providing three user levels (Administrator, Labmanager and Operator) enforcing the strict separation of administrative and analytical tasks. Passwords need to meet user defined requirements with respect to complexity (length, upper/lower case letters, numbers, special characters), prevention of re-use, and expiration. Any login or login attempt is captured in the automatic system audit trail and upon a configurable number of invalid login attempts users are locked. As well, there is an automatic application lock after a configurable activity timeout.

Measurements

Measurements and data evaluation on BRAVO are only possible using method files, which had been officially released (electronic release signature) with pre-set measurement and evaluation parameters. The spectral data (OPUS spectrum file) along with all relevant meta data (timestamp, operator, method file, analysis result, accessory, instrument serial number, software/firmware version, ...) is automatically stored on the BRAVO instrument and captured in the system audit trail.

Electronic Records

Data on BRAVO considered as Electronic Record (ER) are above mentioned OPUS spectrum files (incl. data evaluation), method files as well as audit trails. ERs are stored in a secure manner on BRAVO and no deletion or manipulation is possible by any user independent on the user role. All ERs meet the ALCOA+ principle. The spectroscopy software OPUS enables synchronization/secure data transfer of ERs to a computerized system at first occasion to minimize the risk of data loss (i.e. if the handheld instrument is lost). Using WiFi, a near-instantaneous transfer of data is possible. On the computer system, ERs are managed in the so-called Protected Data Pool, which prevents file deletion and manipulation for operator users on the Windows operating system level.

Electronic Signatures

The OPUS software features true electronic signatures, being managed as part of the user management. Explicitly, users of Administrator role can assign electronic signatures of three categories with individually defined meanings to all users. Electronic signatures strictly follow the requirements set by 21 CFR Part 11 (i.e. first/last name specified for a user account, user ID and password to be specified to initiate a signature session. Furthermore, electronic release signatures enforce a 4-eye principle and can only be applied if a valid electronic review signature from a different user is existing.

Audit Trail

All actions related to the user management, electronic records or relevant parameters changes are captured in the permanent on-board system audit trail. There is no possibility to deactivate audit logging and no possibility modify or delete entries (independent on user role).

Validation Mode

BRAVO's validation mode enforces the following aspects.

- Measurements can only be performed if OQ and PQ performance tests are passed and not expired (compliance to USP and Ph. Eur. performance criteria is ensured).
- Any deletion of files and electronic records is avoided independent on user role. Electronic Records such as routine measurements can be only removed upon a secure transfer to the computerized system. The system audit trail remains permanently on device.
- Method files can be only used for data evaluation upon a valid electronic release signature has been applied following a 4-eye principle.

Independent on user role, the validation mode cannot be deactivated.

System Validation Manual

The system validation manual is the comprehensive documentation covering all aspects of instrument qualification. It includes the validation plan for Design (DQ), Installation (OQ) and Performance Qualification (PQ), which is supported with step-by-step test log forms. Furthermore, it includes relevant certificates and 21 CFR Part 11 compliance documentation, next to various other aspects being covered: OQ/PQ test specifications, maintenance plans, Bruker quality management in software development, relevant manuals, …

The system validation manual is as well basis for the on-site spectrometer qualification services performed by certified and periodically trained engineers.

➲ For further questions on validation topics, please do not hesitate to contact Bruker's validation experts.

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