Application Note AN R532

Advantages of Raman Microscopy and Imaging in Pharmaceutical QC and R&D: an in-depth Review

Introduction

The slightest issues with pharmaceutical products may lead to dangerous and, in the worst cases even fatal scenarios. Therefore, when it comes to the analysis of these pharmaceuticals there is no room for compromise. Production faults in regulated environments must be thoroughly checked and quality control of medicinal products needs to be exact and refined in every regard.

What is the best way to verify the composition of tablets or the distribution of API´s? How can unknown components or contaminations be analyzed spot on, and is it possible to protect myself from infringement by ruthless competitors?

If not already applied, Raman microscopy and imaging offers a straight answer to these questions. It can be employed on the testing of drugs in solid dosage forms, such as tablets and granules, but is also able to differentiate between polymorphous materials and even provides the possibility of non-invasive analysis including depth profiling.

Keywords	Instrumentation and Software
Pharmaceuticals	SENTERRA II Raman Imaging Microscope
API and excipients	OPUS Spectroscopic Software
Particles and inclusions	OPUS/Validation
Distribution and homogeneity	Certified reference standards
Validation	
Pharmacopeia	

Raman microscopy itself delivers a high amount of information on a molecular level, is applicable on pure polymorphic, crystalline and amorphous solids, as well as liquid formulations, sprays and aerosol products. As mentioned before, the identity along with the distribution of APIs, excipients, impurities and contaminations can be verified. Lastly, detection of changes in molecular structure or morphology and evaluation of content uniformity, homogeneity and particle size is feasible with Raman microscopy.

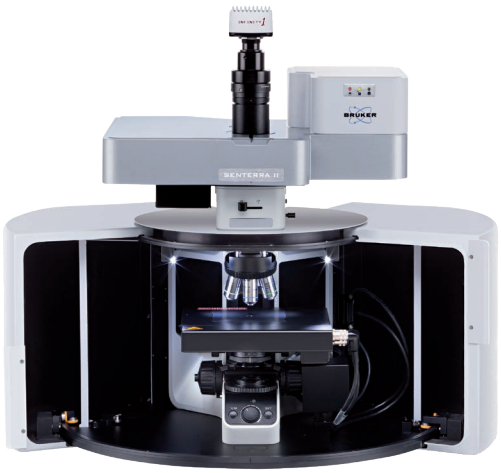
To utilize this powerful spectroscopic method in regulated environments, an equally potent and validated instrument is required and with the SENTERRA II Raman Imaging Microscope (fig.1 top), it is already available.

About the Instrument

As analytical instruments are not only comprised of their technical specifications, but their accessibility for applications as well, the SENTERRA II was designed to ease the user into a self-explanatory workflow. Our goal was to enable even unexperienced operators to pursue their tasks without distractions or overwhelming complexity. We achieved that by implementing infallible automation coupled with a well refined software that allows to control all hardware parameters at the click of a mouse. Laser modes or apertures are almost instantly changed without laying a hand on the device itself and, since calibration is always ensured by SureCAL™ technology, you can rely on unmatched wavenumber accuracy and precision. The user must at no point worry about personal safety, as the apparatus is housed in a class 1 laser safety enclosure and exposition to an active laser during operation is impossible.

The SENTERRA II also incorporates completely automated instrument test procedures according to USP 1120, Ph. Eur. 2.2.48, ASTM E1840 and E2529-06 standards. Moreover, it is fully compliant to GMP, cGMP, GLP and 21 CFR part 11. If required, an optional system validation manual for DQ/IQ/OQ/PQ and validation services is available.

Although the device's unrivalled user convenience takes the spotlight, behind the stage, technical advancements and innovations paved the way. It offers a research grade spectroscopic performance with up to 100 spectra per second, high spectral resolution, the full spectral range and confocal as well as high throughput Raman microscopy capabilities. Fast switching multi laser excitations and minimized fluorescence by an optional 1064 nm excitation, round out its qualities.



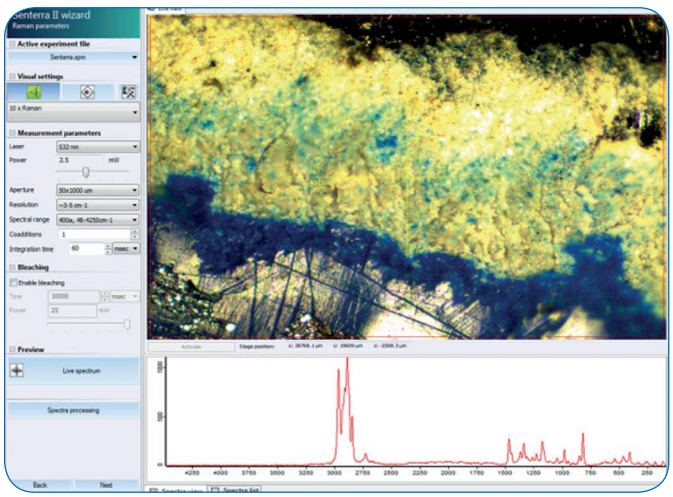


Figure 1: SENTERRA II Raman-microscope (top). Screenshot of the OPUS software while using the SENTERRA II; Wizard (bottom left), live spectrum preview (bottom right) and visual image of the sample.

Using the SENTERRA II means to never worry about the instrument, seize its advantages, be effective and focus on the tasks at hand.

Exemplary Workflow

The analysis is comprehensively guided by the SENTERRA II software assistant. This means only absolute essentials are displayed at each corresponding step, effectively reducing "information noise". First, the specimen is placed on the sampling table, inspected by optical imaging to define areas of interest and an image saved. A parameter window is then opened next to the microscopic sample image along with a live Raman spectrum (fig. 1, bottom).

At this point the user may optimize the Raman measurement preferences, such as selecting the

appropriate excitation lasers and apertures for the analyzed sample. New settings are automatically and instantly adapted by SENTERRA II, allowing to immediately observe the impact of these changes through the live spectrum feature. During the next steps the areas of interest are further specified and then measured. With the obtained data, the OPUS software allows for quick identification of unknown materials via its well-resourced Raman libraries. Furthermore, the Raman data can be used to compile chemical images using a wide range of uni- and multivariate evaluation methods, e.g. integration or cluster analysis.

Example 1: Visualizing the distribution of APIs in a tablet

In the following example a commercially available pain killer tablet was examined with the SENTERRA II to investigate and assess its composition and distribution of contents. After taking a visual image of the tablet, Raman imaging of the surface area was performed using a 532 nm laser (Fig. 2). The Raman image is composed of 360.000 (600 x 600) spectra that cover a wide spectral range from 50 – 4000 cm^{-1}. It was collected with a step width of 22 µm and an integration time of 60 ms per spectrum.

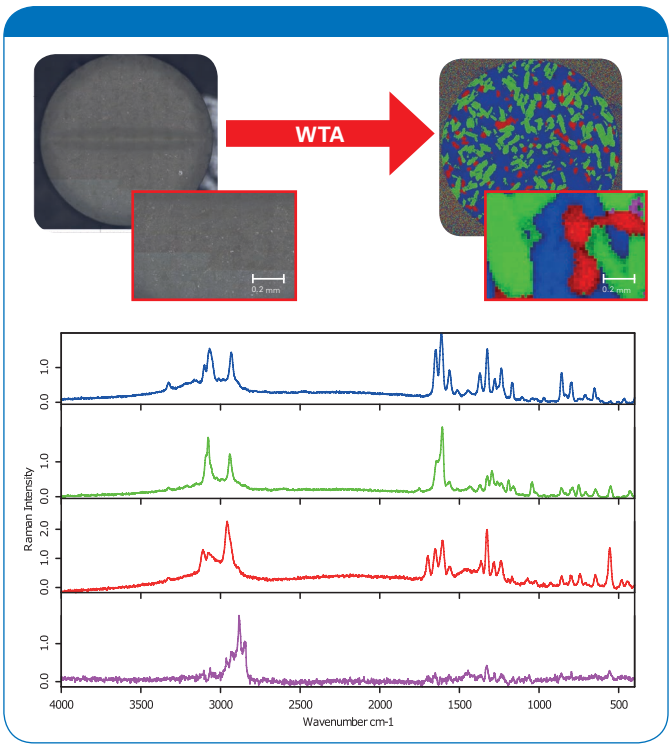


Figure 2: The pain killer tablet and a magnified area of the surface (upper left), and the WTA (winner takes all) plot of the respective contents (upper right): ASA (green), paracetamol (blue), caffeine (red), stearic acid (purple). Corresponding Raman spectra of the single components in their respective colors.

A cluster analysis was performed to structure the extensive data and to approximate the number of components. Thus, at least four ingredient classes were observed and one average spectra of each class was checked against spectral libraries.

Paracetamol, acetylsalicylic acid (ASA), caffeine and stearic acid (as a conglomerate with ASA) were found and verified. To visualize the distribution of APIs, a compound specific spectral range was selected, integrated and a **W**inner-**T**akes-**A**ll (WTA) plot composed. In this representation, each compound is assigned a specific spectral region for integration as well as a color. Out of those, the integral of highest intensity is then determined for each measurement point, which is subsequently highlighted in the color of the respective 'winner'.

Potential ingredient 'hotspots' can then individually be checked for impurities or compared against library spectra to verify the identity. Eventually homogeneity was adequate and the quality of the tablet sufficiently verified.

Example 2: Detection of an unknown intermediate layer in a pharmaceutical pellet

Sometimes the analysis with Raman spectroscopy yields surprising but none the less exciting results. In this case a single grain (1 mm) from inside a pharmaceutical capsule was halved with a razor knife to examine its cross-section. A magnified peripheral view of the pellet can be seen in figure 3. Only three layers of components were expected (coating, API, Substrate), yet remarkably a fourth and unwanted layer between API and coating was observed. When the spectra of the respective layers were checked, it was clear that during production of the granules an unwanted side reaction

occurred, presumably oxidation, leading to a flawed product. Raman imaging was performed on the outer regions of the grain, and the data once again processed by cluster analysis. Component visualization facilitated the location and investigation of the unexpected impurity, and although ultimately the foreign contaminant was not fully characterized, new impulses to improve the production process were gained and later implemented.

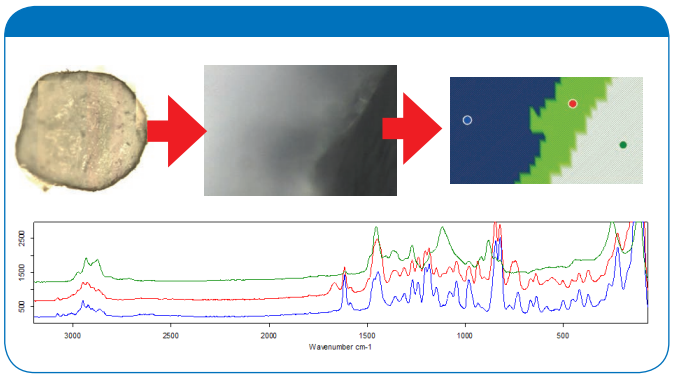


Figure 3: Magnified portrayal of the granule, its outer region and a cluster analysis with points of interest (top); respective color indicated spectra of the highlighted dots in cluster analysis (bottom).

Example 3: Monitoring the thermal interconversion of two polymorphs

Sometimes only one particular crystal modification of a substance is approved for medicinal use, or two polymorphs differ in properties. Ritonavir, an antiretroviral medication used to treat HIV/AIDS, is a nice example in which one of two polymorphs has an inferior oral bioavailability. This means verification of crystallinity is essential to ensure a continually high quality of the product.

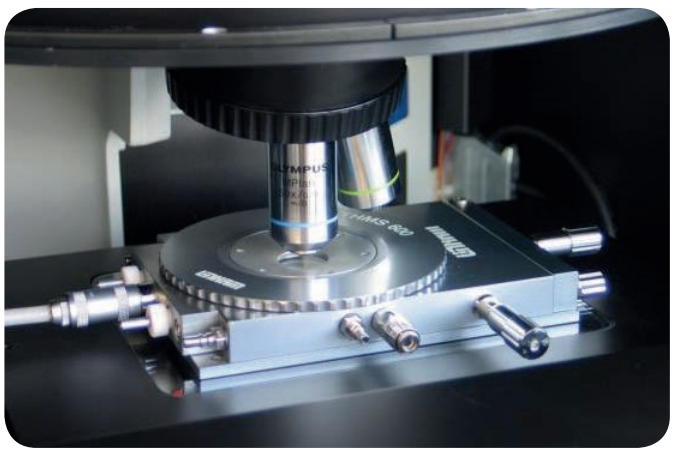


Figure 4: Close-up of the temperature controlled stage for SENTERRA II.

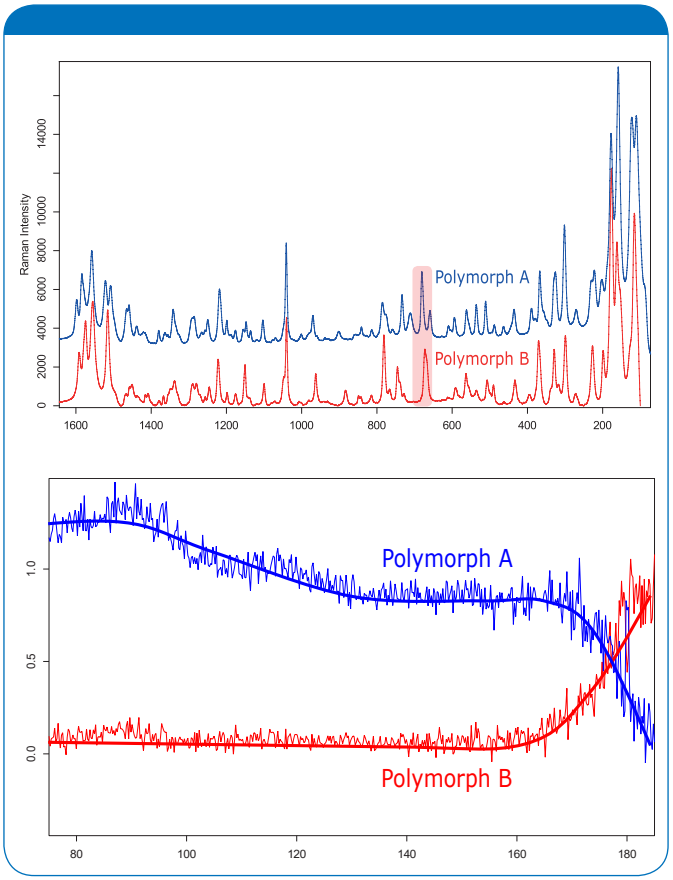


Figure 5: Crucial spectral region for quantification highlighted in red (top); Progression of the polymorphic conversion as a function of temperature (bottom).

In the experiment (fig. 5) the transition between two polymorphs of a generic crystalline sample was examined to pinpoint the optimal temperature for an industrial conversion process. To perform the measurements the SENTERRA II is fitted with a temperature controlled sample stage (fig. 4), that allows to follow the change in morphology live and in high precision. To develop an initial understanding for both polymorphs, they were each investigated by Raman and an appropriate spectral region selected for integration. This was done to establish an indicator for the analysis of the polymorphic ratio (fig. 5; top).

Polymorph A was then set on the sample stage and the temperature ramped with 2°C per min while integration time was set to 1 sec per spectrum. At the beginning, primarily only polymorph A can be detected but as temperatures rise the conversion commences slowly at 160°C and is completed at ~185°C (fig. 5; bottom). By monitoring the formation of polymorph B, it was possible to determine the most efficient conditions for an economical production.

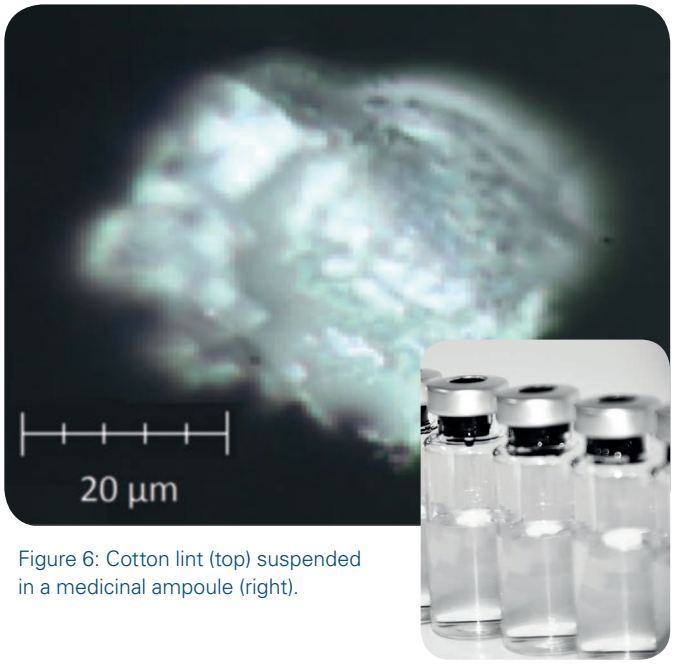


Figure 6: Cotton lint (top) suspended in a medicinal ampoule (right).

Example 4: Non-invasive analysis

Liquid formulations demand especially high quality standards, since particles or other insoluble contaminants may lead to critical complications during drug administration. In this regard, a practical application is the non-invasive analysis of a foreign substance in a sealed and liquid filled glass vial. Figure 6 shows a microscopic image of a white voluminous particle floating in the flask.

Through careful Raman measurements the particle was selectively analyzed within the ampoule, the obtained spectra checked against spectral libraries and the unwelcomed fleck identified as cotton lint. Yet these application possibilities do not stop at glasswear, since every material behind transparent packaging can be analyzed by this method, e.g. adhesions within syringes, or foreign inclusions in gel capsules, blisters and foils.

Conclusion

These examples thoroughly emphasize the benefits of Raman microscopy and imaging by the SENTERRA II when utilized in pharmaceutical applications. It offers an intuitive smooth workflow, comprehensive validation, research grade spectroscopic performance, compact size and the highest level of automation. Permanently calibrated by SureCAL™ technology the SENTERRA II allows the user to push the limits of Raman imaging and microscopy and make analysis not only easier but also more productive than ever before.

Working with the SENTERRA II means to:

- seize its advantages -
- be effective -
- and focus on the tasks at hand -

Bruker Optik GmbH
Ettlingen · Deutschland
Phone +49 (7243) 504-2000
Fax +49 (7243) 504-2050
info.bopt.de@bruker.com

Bruker Optics Inc.
Billerica, MA · USA
Phone +1 (978) 439-9899
Fax +1 (978) 663-9177
info.bopt.us@bruker.com

Bruker Shanghai Ltd.
Shanghai · China
Phone +86 21 51720-890
Fax +86 21 51720-899
info.bopt.cn@bruker.com

www.bruker.com/optics