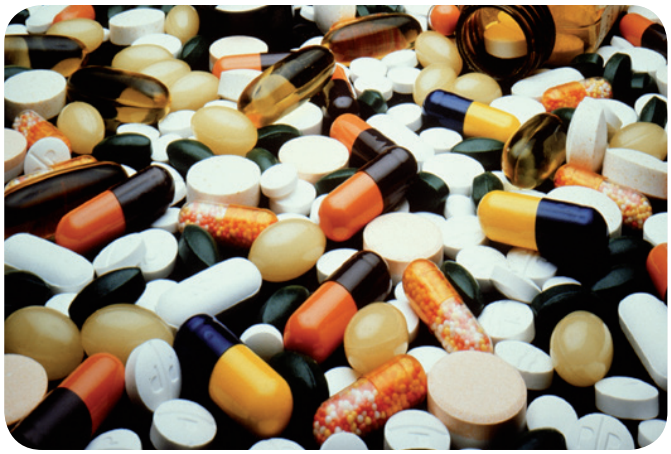

Application Note AN M119

Microanalysis in Pharmaceutical Product Development and Trouble-Shooting

The demand on the quality of pharmaceutical products is very high. Raw and packaging materials as well as intermediate and final products must contain the correct components and need to be free from contaminations. In quality control analytical methods like Mid- and Near-IR spectroscopy and others allow to verify an overall correct identity and composition of the sample. However, contaminants like particles in liquid formulations or inclusions in tablets are often extremely small and cannot be detected selectively by such macroscopic methods. Therefore further analytical methods are needed to determine the accurate chemical nature of the particle or inclusion – valuable information that allows to find the source of contamination.

The FTIR microscope LUMOS II (fig. 1) allows to measure smallest structures and to determine their chemical composition. LUMOS II is a stand-alone system that is very easy to use due to its full automation and intuitive analysis software. Furthermore its design is very compact and space saving. Due to these features the LUMOS II is very suitable for the use in routine analysis.

Keywords	Instrumentation and Software
Pharmaceutical products	LUMOS II FTIR microscope
FTIR microscopy	OPUS spectroscopic software
Quality control	OPUS/SEARCH
Trouble shooting	OPUS/3D
Particles and contaminants	ATR-COMPLETE spectral library

Fig. 1 FTIR microscope LUMOS II: Its easy handling results in the quick results required for effective trouble-shooting

In addition the LUMOS II generates precious information in the field of pharmaceutical development and reverse engineering. Measurements with a local resolution in the micrometer range allow characterizing the composition of a tablet or lyophilisate. Mapping measurements on the sample reveal the distribution of individual components, e.g. the API, excipients and even different polymorphic forms of the same compound. Also the different layers of complex composite materials like multilayer films used for packaging can be identified.

Fig. 2: Pharmaceutical tablet with contamination (yellow)

Identification of an inclusion inside a pharmaceutical tablet

Pharmaceutical tablets have to be free from inclusions. If an inclusion is detected e.g. by visual inspection its chemical nature has to be determined to raise the chances finding its origin. Figure 2 shows a white tablet with a yellow spot inside.

To discriminate the inclusion from the general matrix of the tablet a series of positions was measured on the yellow spot and next to it. Using the fully automated ATR (Attenuated Total Reflectance) mode of the LUMOS II the region of interest was analyzed without any sample preparation; the tablet just had to be fixed in a micro vice on the microscopic stage. Guided by the software microscopic images of the region of interest were taken and measurement positions defined before the measurement was performed by the LUMOS II automatically. An area of 10x10µm per spot was measured with the spectrum acquisition taking 10 seconds, respectively. Figure 3 shows the visual microscopic image of the inclusion inside the tablet together with the measurement positions. The spectra are colored according to their position. It is obvious that the spectra measured on the inclusion (blue) have a much different signature than the ones on the general matrix of the tablet (red).

Similarity library spectrum / measured spectrum

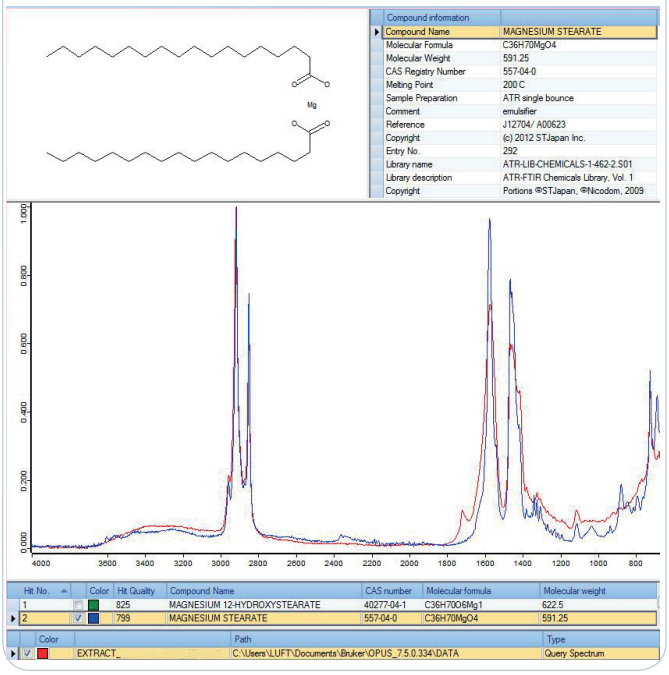

Fig 4: Clear identification of the inclusion as magnesium stearate by library search

The identification of the inclusion as magnesium stearate was achieved within a few seconds by searching the comprehensive spectral library ATR-COMPLETE which contains > 26.000

Result of IR-microscopic measurement

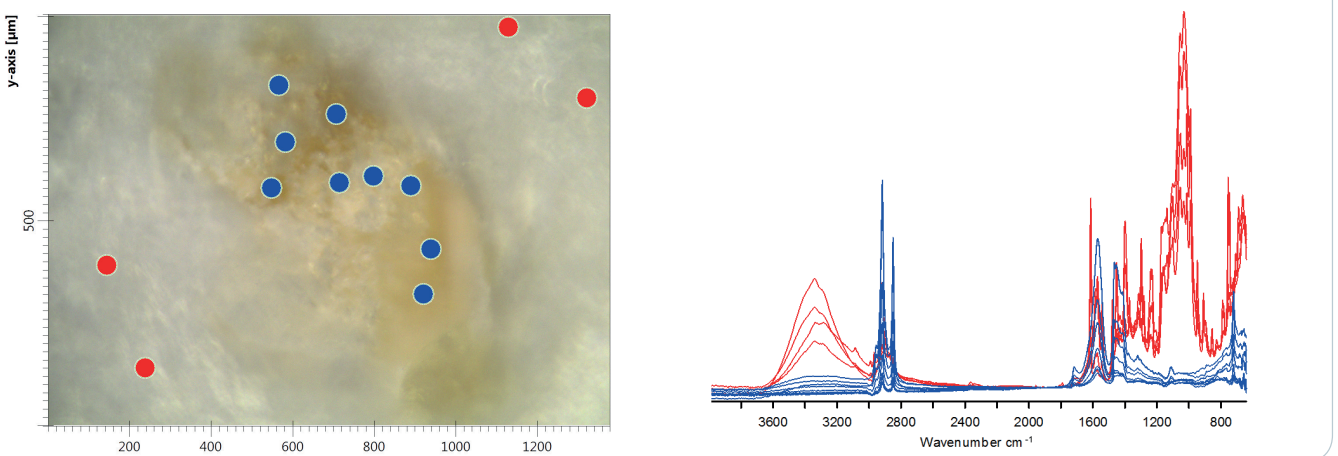

Fig 3: IR-microscopic mapping on pharmaceutical tablet with unwanted yellow inclusion. The spectra on the inclusion (blue) show a different signature compared to those from the tablet matrix (red)

spectra of various substances. Fig. 4 shows the high similarity of the library spectrum with the measured spectrum.

Determination of the chemical identity of a particle

Liquid sterile formulations must be free from all visible and even smaller particles. Unwanted particles might originate from the manufacturing equipment, the personnel or the used packaging material. Also unwanted precipitation of the active ingredient and excipient might result in particles. Hence, the chemical composition of potential particles covers a wide range including plastic and rubber fragments, cellulose fibers, glass, metal or biological material like protein.

For further analysis particles are isolated from the liquid formulation using a gold filter (Fig.5) which can be used directly as substrate for the IR-microscopic measurement. Figure 6 shows the microscopic image of several fibers that were filtered from the liquid formulation of a protein drug. The spectra were measured without further sample preparation directly on the fiber bundle and on the filter substrate next to it with the LUMOS II by applying the ATR-technique. Whereas the spectra on the filter are typical for the protein drug (amide I at 1640 cm^{-1} and amide II 1550 cm^{-1}; not shown) the fiber has a strong double peak around 1200 cm^{-1}. This spectrum could be identified to originate from Poly(tetrafluoroethylene) (Teflon) by searching the ATR-COMPLETE spectral library (Fig.6). The result clearly shows that the particles are not a precipitate from the formulation but could stem from a filter used during production or from packaging (e.g. abrasion from a stopper).

Fig 5: Gold filter for the isolation of particles from liquid formulations

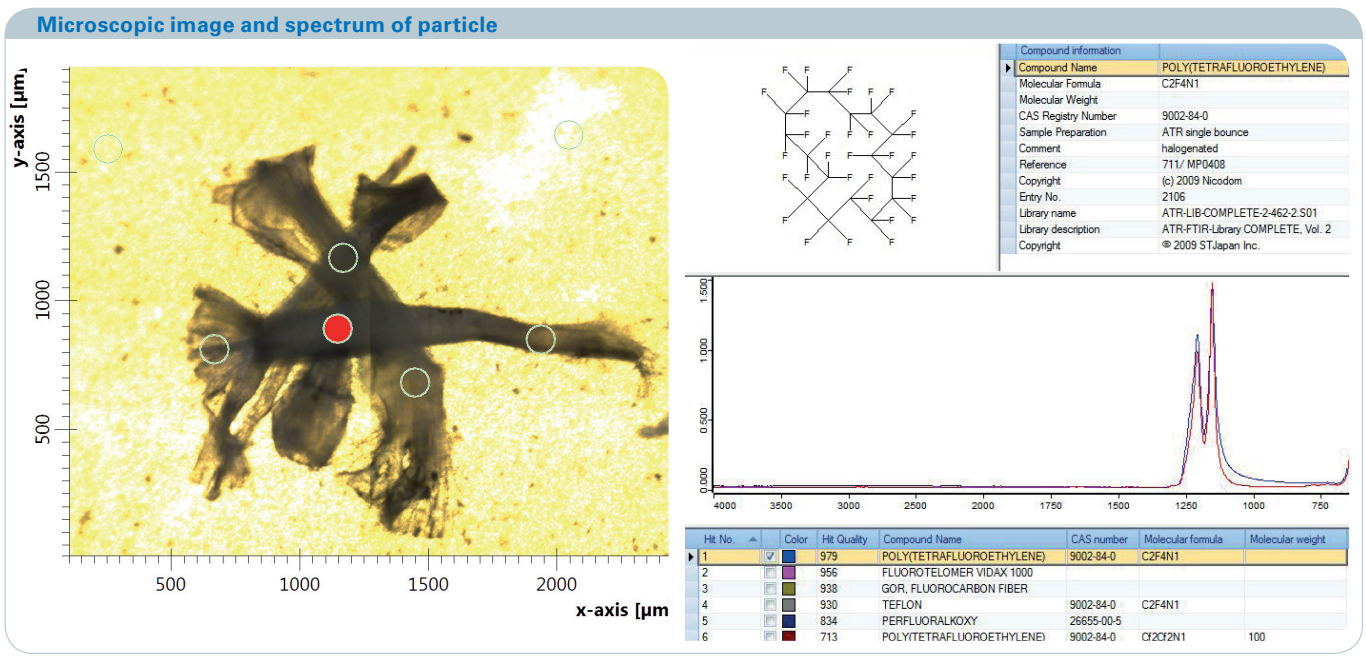

Fig 6, left: Microscopic image of a bundle of fibers on a gold filter separated from a liquid biopharmaceutical. Right: Identification of the spectrum on the indicated measurement position as Poly(tetrafluoroethylene) (Teflon) by library search tablet covering an area of 500x325µm. The spatial resolution of 25x25µm (indicated by red rectangles) is defined by the operator using an automated physical aperture which is integrated in the LUMOS II

Analysis of the distribution of API and excipients in a pharmaceutical tablet

The quality of a pharmaceutical tablet is not only defined by its components but also by their distribution inside the tablet. As an example a protective layer around the tablet as well as the homogeneity of the active pharmaceutical ingredient (API) distribution defines where and at which speed the API is released inside the body. IR-microscopic mapping measurements create chemical images of the API and the excipients distribution.

In this example an fully automated ATR-mapping on the cross-section of an pharmaceutical tablet of the anti-inflammatory drug ibuprofen was performed using the LUMOS II. An area of 500x325µm was measured with a spatial resolution of 25x25µm (Fig 7).

Apart from the active pharmaceutical ingredient ibuprofen the excipients lactose, microcrystalline cellulose and sodium dodecyl sulfate are present in the tablet. To determine their distribution inside the tablet a linear combination of previously measured pure substance spectra was performed to explain the composition of each individual mapping spectrum. The calculation was done fully automatic using the OPUS spectroscopic software.

Rectangular mapping grid

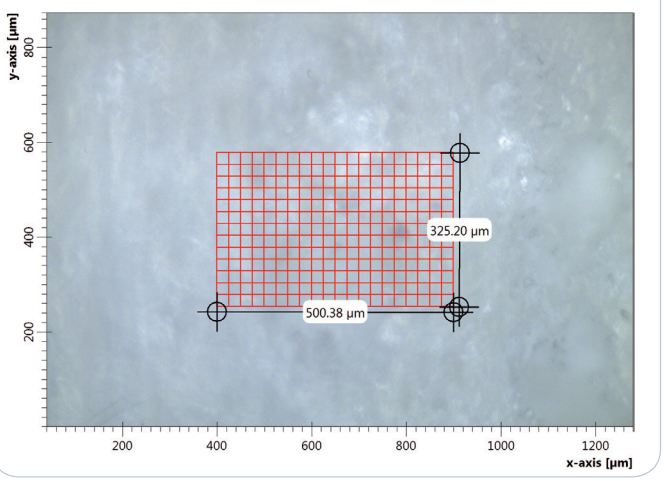

Fig.7 Rectangular map on the cross section of a pharmaceutical tablet covering an area of 500x325µm. The spatial resolution of 25x25µm (indicated by red rectangles) is defined by the operator using an automated physical aperture which is integrated in the LUMOS II

Chemical images

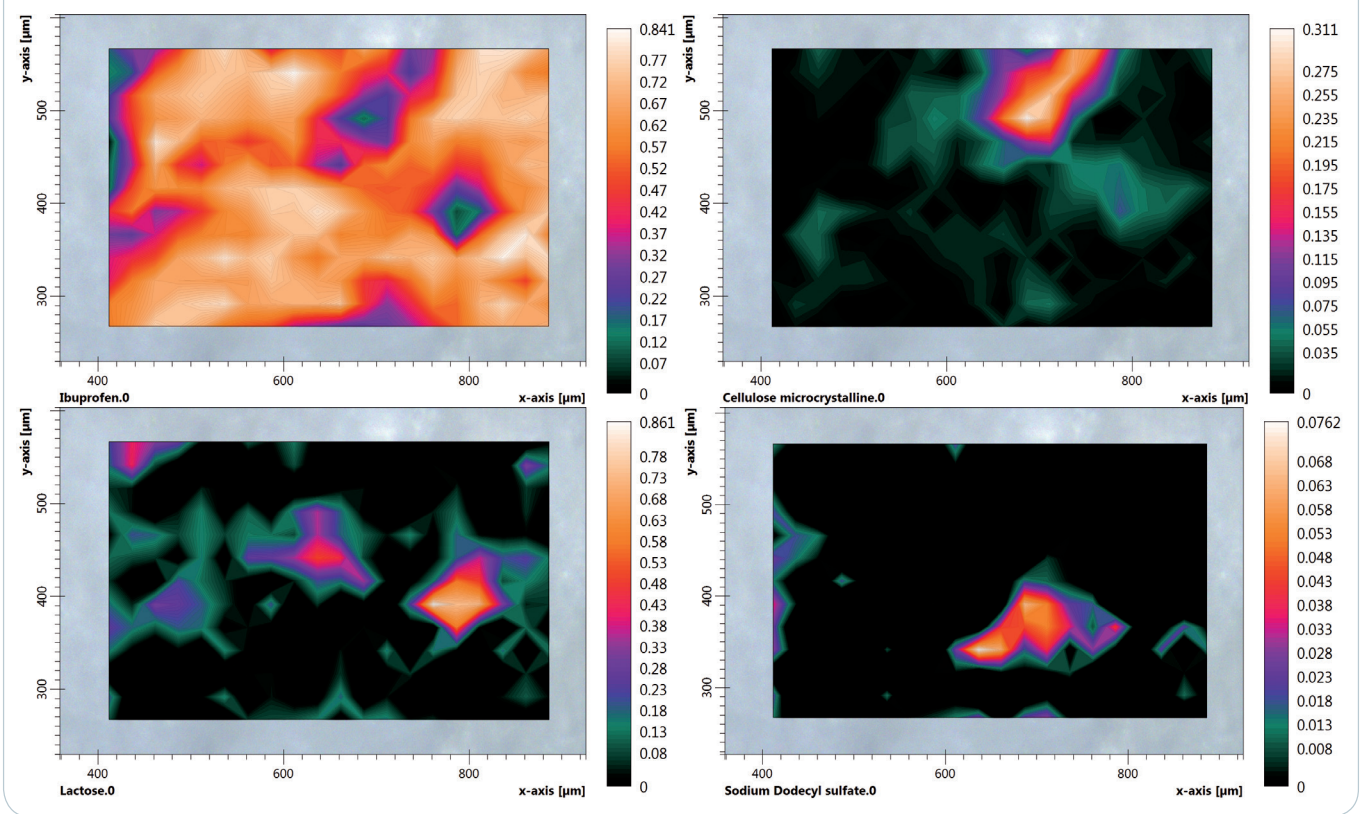

Fig.8 Chemical images of the distribution of different compounds in a pharmaceutical tablet: ibuprofen (upper left), microcrystalline cellulose (upper right), lactose (lower left) and sodium dodecyl sulfate (lower right)

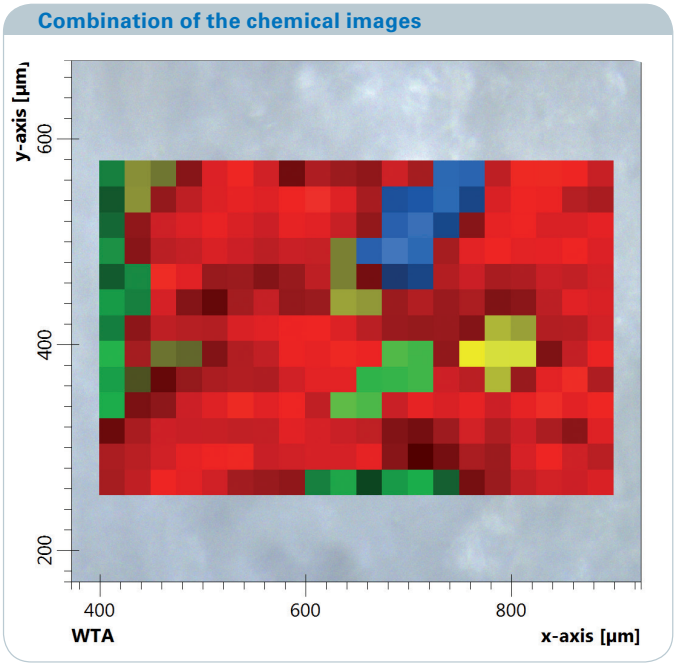

Fig.9 Combined chemical images of the distribution of different compounds in a pharmaceutical tablet: ibuprofen (red), microcrystalline cellulose (blue), lactose (yellow) and sodium dodecyl sulfate (green).

The chemical images in figure 8 visualize the composition of the tablet at each measurement spot using a color scheme ranging from black (no contribution of component) to bright orange (high contribution of component).
Combination of the chemical images shows which part of the tablet is dominated by which component (figure 9).

Summary

IR-microanalysis is an extremely powerful and valuable technique to effectively track the source of product contaminations found in routine quality control. Furthermore the spatially resolved analysis of pharmaceutical products like tablets or lyophilisates provides insight in their composition and homogeneity. This knowledge helps pharmaceutical research and development to optimize products and learn about the competition.
Using the compact FTIR microscope LUMOS II even inexperienced users are able to perform an IR-microscopic analysis with minimal training. It's full automation and intuitive software interface result in a user comfort that will save your working time.

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