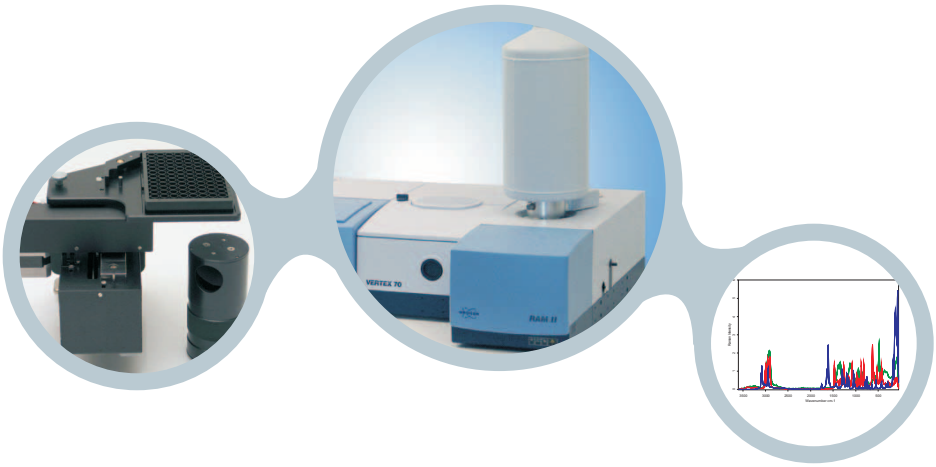

Application Note AN # 514

Fully automated Raman measurements of pharmaceutical polymorphs and formulations

Introduction

In the past years pharmaceutical industry has recognized the need for high-throughput screening techniques for drug development as well as for the discovery process of new drugs[1]. The identification and classification of solid state forms requires high standards of automation. The need to understand the behaviour of solid state forms derives from the fact that the crystal structure of active pharmaceutical agents (API's) has a high impact on physical properties such as long term stability or bioavailability. Raman spectroscopy is a useful method to study the polymorphism on the molecular level during the crystallisation process of solid forms.

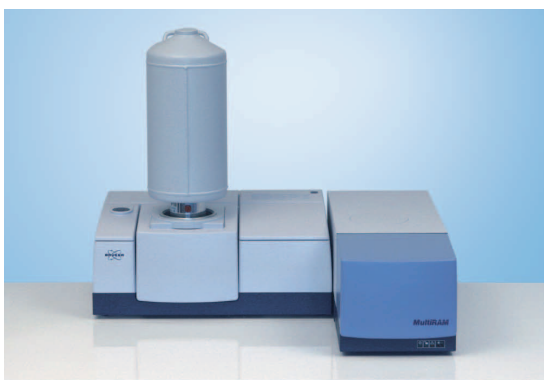

Fig. 1 FT-Raman spectrometer MultiRAM

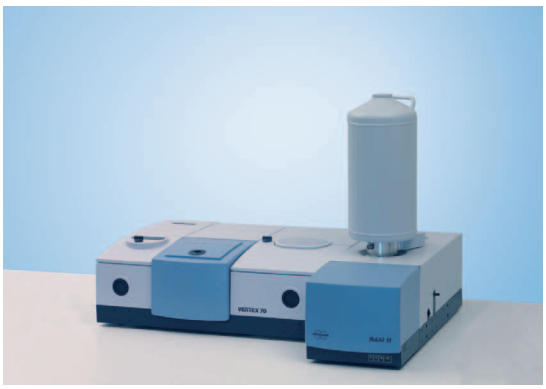

Fig. 2 FT-Raman module RAMII attached to a VERTEX FT-IR spectrometer

Other typical applications of Raman spectroscopy are the identification of polymorphs in the final formulation, homogeneity studies, quantifications of active ingredients or polymorphs as well as stability studies depending upon temperature, humidity or other parameters[2,3,4].

A suitable analysis method for high throughput screening should offer:

- No or little sample preparation
- Application of standard high throughput formats (well plates)
- Informative and reliable data with high information content for validated use
- Ability to detect small amounts of samples
- No interference or change of samples by the analysis

Fig. 3 HTS-Raman accessory for automated measurements of multiple samples

method

Raman spectroscopy is one technique which meets the above requirements. Due to its non contact and non destructive character Raman spectroscopy is applicable for the investigation of polymorphs, salt forms, solvates and other pharmaceutical substances. This paper focuses on qualitative and quantitative pharmaceutical applications of Raman spectroscopy in combination with a high throughput screening (HTS) accessory.

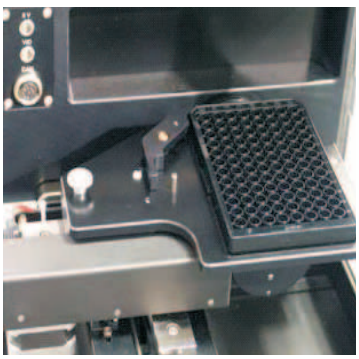

Fig. 4 HTS-Raman accessory mounted in the FT-Raman sample compartment

Instrumentation

The HTS-Raman accessory shown in fig. 3 can be used in combination with either the stand-alone FT-Raman spectrometer MultiRAM or the FT-Raman module RAM II (fig. 1 and fig. 2). The accessory uses standard 96 wells micro plate formats. Samples such as pharmaceutical powders are loaded into the well plate without any sample preparation. The analysis is performed through the glass bottom of the well plate (fig.4) avoiding the need for time consuming focusing to the sample area. The filling height of each well is not a crucial issue as compared to measurements from the top.

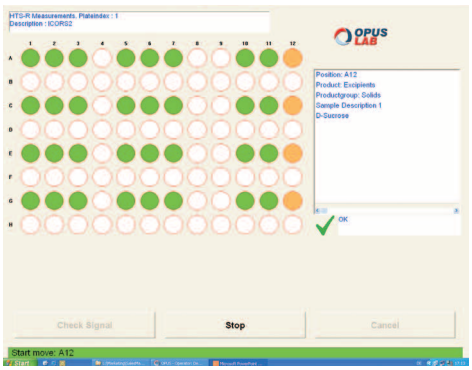

Fig. 5 Definition of sampling positions in OPUS/LAB

Further the use of a NIR excited FT-Raman spectrometer provides additional benefits for high throughput measurements. The virtual absence of fluorescence by using long wave length excitation at 1064nm quite frequently are yielding low noise spectra allowing short acquisition times.

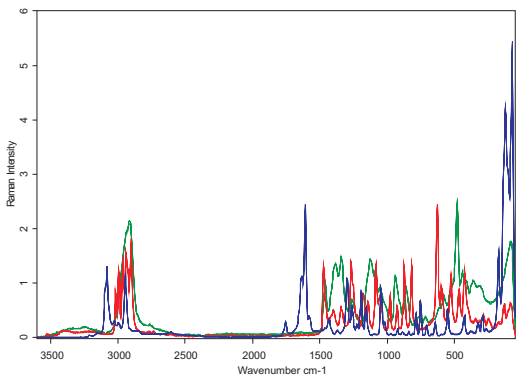

Fig. 6 FT-Raman spectra of Acetyl Salicylic Acid, Fructose and Starch (3 measurements for each substance)

FT-Raman spectrometers offer high spectral resolution (< 1cm^{-1}) if required. The spectral resolution of a spectrometer based on Fourier transform technology can be precisely adapted for each measurement campaign, saving unnecessary long scan times.

Furthermore it is quite common in pharmaceutical screening in the R&D department of companies to record the entire spectral range rather than a fraction of it. Fourier Transform Raman spectrometers are inherently collecting the entire spectral range independently from

the required spectral resolution, which overall results in time savings.

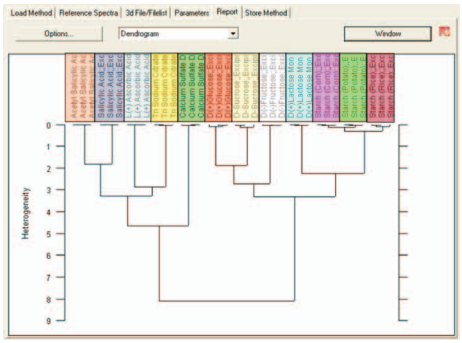

Fig. 7 Cluster analysis (dendrogram) of pharmaceutical substances showing the capability of FT-Raman for classification of those compounds

Substance identification

A typical application for High Throughput Screening is the identification of polymorphs or other pharmaceutical products. Spectra of measured samples are compared to a set of reference spectra. For the measurement campaign given in this paper 12 different APIs and excipients have been measured fully automated by means of the HTS-Raman accessory. The user interface OPUS/LAB assists to setup the measurement of multiple samples quickly and easily (fig.5). As an example the FT-Raman spectra of acetyl salicylic acid, fructose and starch are presented in fig.6.

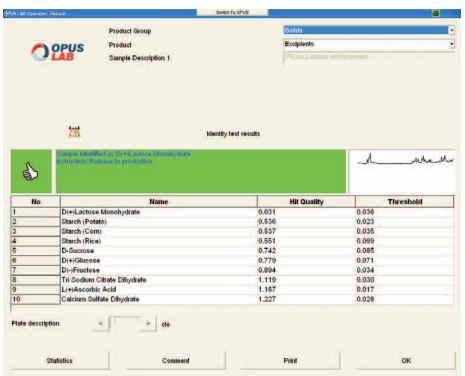

Fig. 8 Positive substance identification in OPUS/LAB

Spectral comparison is done by calculating a value that reflects the amount of spectral differences or the similarity of spectra such as Euclidean or Mahalanobis distance. The aim when setting up a identification library is to separate groups of substances uniquely from each other as represented in the cluster analysis shown in fig. 7. Even very similar compounds such as different sugars (sucrose, fructose and glucose) or starches from corn, potato and rice can be clearly classified in this way. Fig.8 shows the positive and distinct identification of a newly measured sample by comparison with a reference library of 12 APIs and excipients.

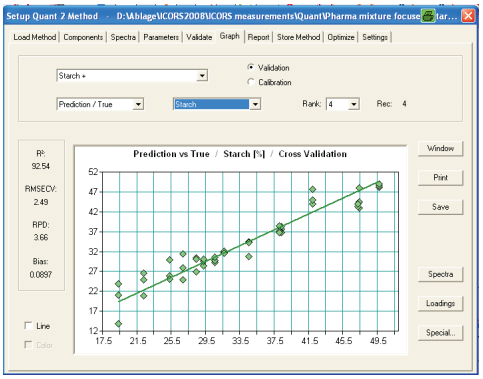

Fig. 9 Calibration curve for starch based on 15 reference samples with varying amounts and PLS algorithm

Quantification

Besides qualitative also quantitative evaluations play an important role when Raman spectroscopy is used in pharmaceutical industry. Basically both univariate and multivariate methods can be used for development of quantitative models. For the quantitative study in this paper chemometric algorithms have been used: 15 pharmaceutical mixtures consisting of varying amounts of 4 different compounds have been measured for setting up the quant methods. In fig. 9 and 10 the corresponding calibrations for starch and cellulose are presented. The average prediction errors (RMSECV) are found to be 1.4 and 2.5% respectively. The reduction of the error prediction can be achieved by providing more homogeneous samples and/or mapping a larger area within one well. Fig. 11 shows the quantitative prediction of an unknown

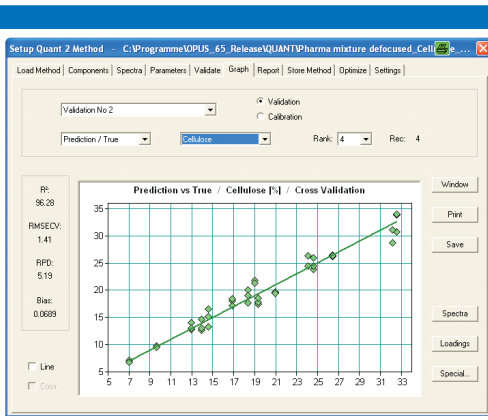

Fig. 10 Calibration curve for cellulose based on 15 reference samples with varying amounts and PLS algorithm

mixture for cellulose and starch by using above calibrations.

Summary

FT-Raman spectroscopy in combination with a high throughput screening accessory is the ideal tool for automated measurements of polymorphs or other pharmaceutical products. Due to the availability of powerful and intuitive software packages a rapid processing of even huge amounts of spectroscopic data is possible nowadays easily and quickly.

1. Morisette SL, Almarsson OE, Peterson ML, Remenar JF, Read MJ, Lemmo AV, Ellis S, Cima MJ, Gardner CR. J. Raman Spectrosc. 2004; 56: 275
2. Kachrimanis K, Braun D, Griesser UJ. J. Pharm. Biomed. Anal. 2007; 43/2: 407
3. Schmidt AG, Wartewig S, Picker KM. J. Raman Spectrosc. 2004; 35: 360
4. Auer ME, Griesser UJ, Sawatzki. J. J. Mol Struct. 2003; 661-662: 307

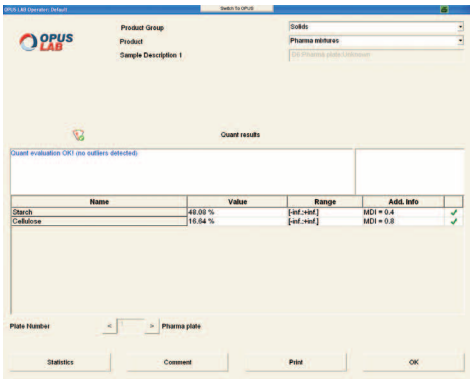

Fig. 11 Product quantification in OPUS/LAB

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