High-throughput Screening Application Note

Assessing Ritonavir Crystallinity Using Low-Frequency Raman Spectroscopy

The Challenge

Polymorphism is critical in pharmaceuticals, affecting properties like solubility and bioactivity. A prominent case involved ritonavir capsules in 1998, triggering a recall due to a second crystalline form with reduced solubility. Ritonavir, treating HIV/AIDS and COVID-19, was reformulated as an amorphous solid dispersion (ASD) via hot-melt-extrusion (HME) after this discovery. ASD stability depends on factors like the glass transition temperature and water vapor sorption. Monitoring crystallinity in ritonavir tablets post-market is crucial. Traditional methods involve complex sample preparation, with X-ray powder diffraction (XRPD) being error-prone. Storage conditions, including temperature and humidity, play vital roles in ensuring the long-term stability of ASDs.

Traditional Solutions

Traditionally, crystallinity and polymorphism analysis is performed using X-ray powder diffraction (XRPD), differential scanning calorimetry, and scanning electron or polarized light microscopy (SEM or PLM). Among these, XRPD allows development of robust quantitative and semiquantitative methods but requires tedious sample preparation and is prone to errors due to orientation effects and sample displacement.

Attalon Solutions

Advanced solid-state pharmaceutical characterization methods include terahertz spectroscopy and low-frequency Raman (LFR) spectroscopy, offering sensitivity akin to XRPD with minimal sample preparation. LFR spectroscopy, combined with variants of Raman spectroscopy, has been employed in diverse studies, such as polytype identification, cocrystal monitoring, chiral purity assessment, and amorphous phase characterization. Berzins et al. conducted a comprehensive review on pharmaceutical LFR applications, emphasizing its non-destructive and rapid solid-state characterization. In a specific study, Raman methods demonstrated similar sensitivity to XRPD but significantly reduced analysis time, requiring 231 hours for XRPD versus 35 minutes for Raman in a storage study. Additionally, a semiquantitative transmission LFR method efficiently assessed crystallinity in ritonavir tablets without sample preparation.

Application Field

Raman, High Throughput Screening, Polymorphs, Crystallinity, Polarized Light Microscopy, Pharmaceuticals, Biopharma

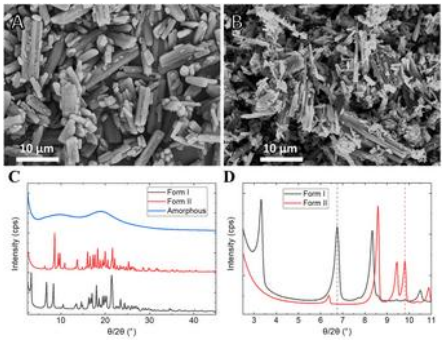

Figure 1. *SEM images of secondary-form I (A) and primary-form II (B) reference standards of ritonavir. Form I has a monoclinic crystal system whereas form II is orthorhombic. X-ray powder diffraction patterns of amorphous and crystalline (form I and form II) ritonavir in the range of 2.545° (C). Unique and characteristic peaks of each form were identified at low angles (D). Gray and red dashed lines correspond to peaks used in quantification based on height.*

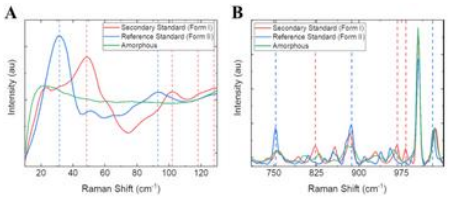

Figure 2. *LFR (A) and MFR (B) spectra of amorphous (green), form I (red), and form II (blue) ritonavir.*

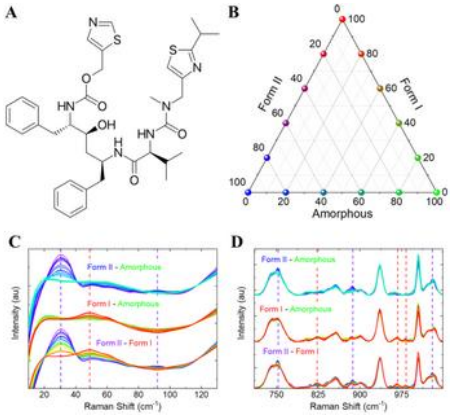

Figure 3. *(A) Molecular structure of ritonavir. (B) Ternary plot demonstrating the LFR and MFR model samples. LFR (C) and MFR (D) spectra of model samples color-coded to represent each phase.*