Identify, count, and size APIs and their polymorphs with Hound

Introduction

Polymorphism is a common occurrence in the development of small molecule active pharmaceutical ingredients (APIs). While polymorphs maintain the same chemical identity, they differ in crystal structure. Differences in crystal structures can significantly alter the bioavailability, stability, solubility, and/or dissolution rate of the API. Polymorphs can form during discovery, development, or manufacturing so it is crucial to be able to quickly screen and determine which polymorphs are present.

Raman spectroscopy is an alternative approach to X-ray powder diffraction (XRD), FT-IR, and near-IR spectroscopy to identify APIs and their polymorphs. Most APIs are aromatic or conjugated systems with delocalized electron systems that produce strong Raman scattering and require little sample. When comparing polymorphs, differences in Raman scattering occur due to molecule-molecule interactions in the crystal structures resulting in distinct vibrational modes that make each polymorph easily identifiable. Raman is a sample preparation free method that can readily identify amorphous and small crystals. When paired with a microscope, Raman can measure the distribution of polymorph crystals.

Hound combines automated microscopy and Raman spectroscopy to identify visible and subvisible particles across a wide range of chemical compositions (Figure 1). Automated microscopy rapidly counts and sizes all particles in a sample. Identified particles are then analyzed with image-directed Raman spectroscopy. Raman spectroscopy is used to chemically fingerprint organic and inorganic particles which are automatically matched to a built-in, customizable reference database for identification. Hound makes screening polymorphs simple by automatically counting, sizing, and identifying polymorphs in a mixture with Raman spectroscopy.

Figure 1: Hound counts and identifies the composition of visible and subvisible particles.

In this application note, Hound was used to perform automated Raman spectroscopy to identify the composition of an unknown mixture that potentially consisted of two compounds each with two polymorphs. Hound automatically imaged particles from the powder sample to determine the particle count and size distribution followed by automated Raman spectroscopy to identify the makeup of the mixture.

Methods

Reference spectra collection

The Raman spectra for two purified polymorphs of olanzapine and indomethacin (Figure 2), were collected on Hound to create a custom reference library. For the purpose of blinding the study, olanzapine was labeled as Compound A and indomethacin was labeled as Compound B. Each sample potentially contained two polymorphs.

Sample preparation

Compounds A and B were mixed at unknown ratios to create a mixed sample potentially con-

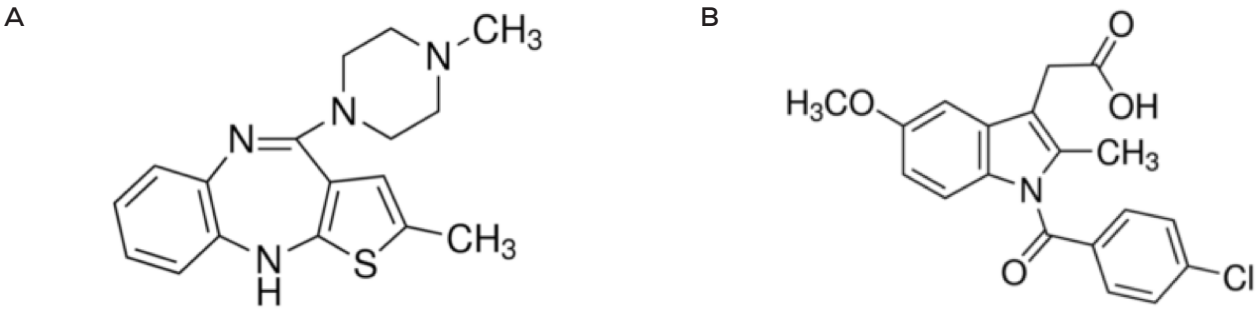


Figure 2: **(A)** The chemical structure of Compound A, olanzapine. **(B)** The chemical structure of Compound B, indomethacin.

taining both polymorphs of both compounds. A thin layer of the powder was spread onto a 0.8 µm filter round for automated Raman analysis.

Automated Raman analysis

The filter round was automatically analyzed on Hound by scanning 64 fields of view (500.5 µm X 500.5 µm) to image and analyze all particles >2.00 µm. Raman analysis was conducted at 80% laser intensity for 35 seconds per measurement.

The Raman spectrum from each particle was compared to a custom reference library containing the purified polymorphs of each compound. A match rank between the sample and the reference spectra was calculated by multiplying the Pearson correlation by 1,000. A match rank greater than 700 (out of 1,000) was considered a high-quality match.

Results

Identifying Polymorphs with Raman

Polymorphs of each compound showed significant differences when analyzed with Raman on Hound. Raman spectra from Compound A Polymorphs 1 and 2 show significant differences at multiple wavenumbers, including 335 cm^{-1}, 440 cm^{-1}, 825 cm^{-1}, and 1020 cm^{-1} (Figure 3A). These spectral differences between the polymorphs of Compound A allow Hound to distinguish between Polymorph 1 and Polymorph 2 particles. Likewise, Hound distinguished between the two polymorphs of Compound B due to significant differences in the Raman spectra at multiple wavenumbers, such as 405 cm^{-1}, 700 cm^{-1}, 845 cm^{-1}, 970 cm^{-1}, and 1,315 cm^{-1} (Figure 3B).

Raman Analysis of an Unknown Mixture

A full scan of the unknown sample resulted in the identification of 242 particles, 227 (~94%) of which were API particles. The mixed sample was comprised of Compound A, Compound B and a

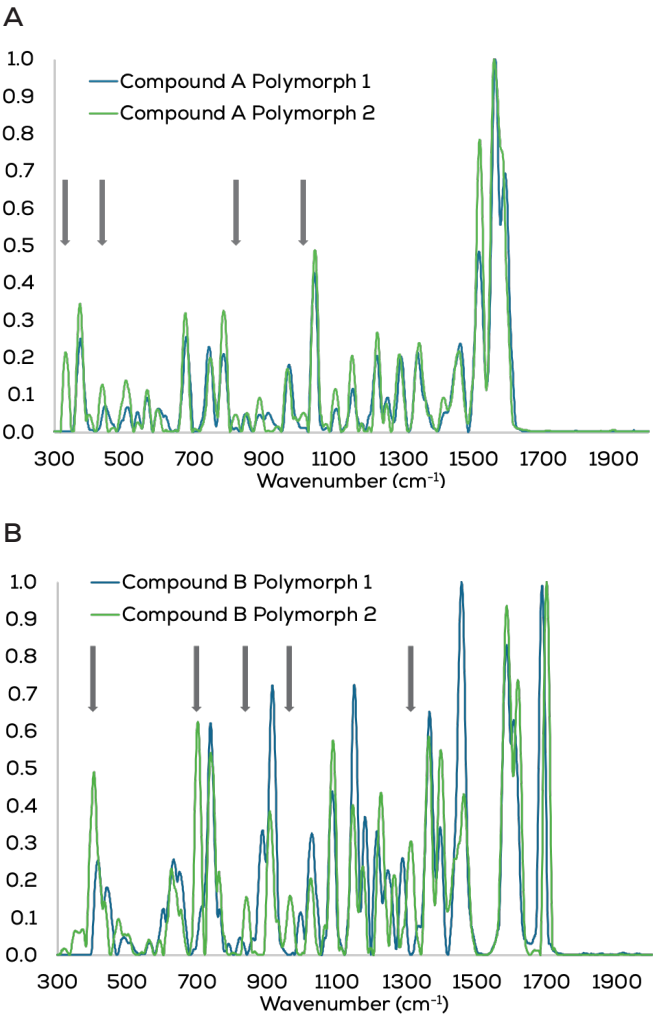


Figure 3: **(A)** The collected reference spectra for the two purified polymorphs of Compound A. Arrows indicate key differences in the spectra of the polymorphs. **(B)** The collected reference spectra for the two purified polymorphs of Compound B, with arrows indicating key differences.

Reference Match	Total Count	2 - <5 µm	5 - <10 µm	10 - <25 µm	25 - <50 µm	50 - <100 µm	≥100 µm
Compound B Polymorph 2	218	112	7	19	18	20	42
Compound A Polymorph 1	6	2	1	2	1	0	0
Compound A Polymorph 2	3	0	1	1	0	1	0
Petroleum Jelly	1	1	0	0	0	0	0
Glass	1	1	0	0	0	0	0
Unknown	1	1	0	0	0	0	0
Fluorescence	12	11	1	0	0	0	0
Total analyzed	**242**	**128**	**10**	**22**	**19**	**21**	**42**

Table 1: The particle count and distribution of the unknown mixture. Particles were automatically counted, then chemically identified with Raman spectroscopy.

few contaminants (Table 1). A majority of the API particles, 218 out of 227 particles (~96%), were identified as Compound B Polymorph 2 (Figure 4). Both polymorphs of Compound A were present. Six particles (~3%) were identified as Compound A Polymorph 1 (Figure 5) and three particles (~1%) identified as Compound A Polymorph 2 (Figure 6).

Glass and petroleum jelly were identified as contaminants in the mixture (Table 1). With the ability to identify polymorphs and their distribution, it is possible to compare preparations and lots to determine both chemical purity and the presence and distribution of polymorphs.

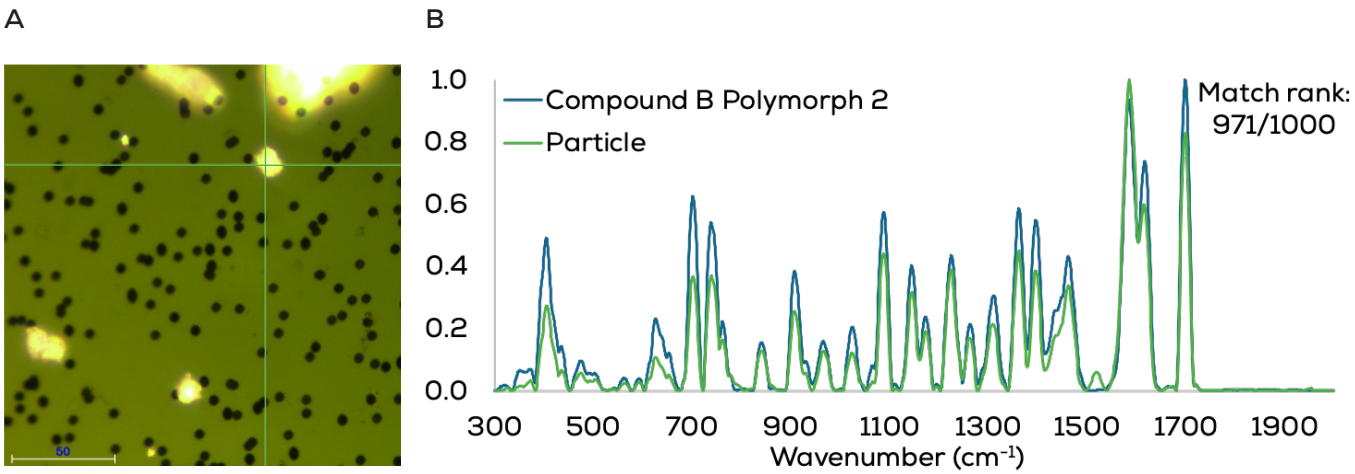
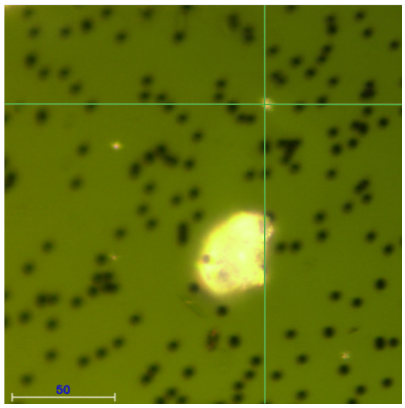


Figure 4: **(A)** Compound B Polymorph 2 particle identified after automatically scanning the filter round. **(B)** The Raman spectrum of the Compound B Polymorph 2 particle (blue) was matched to the custom reference spectrum for Compound B Polymorph 2 (green) with a rank of 971/1,000.

A



B

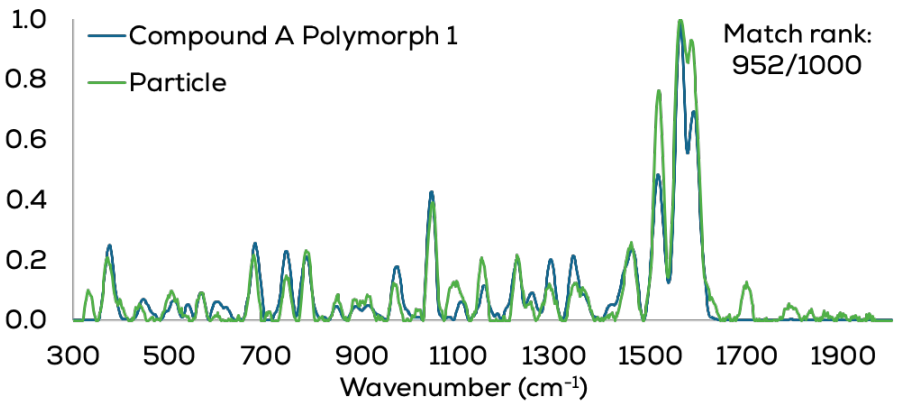
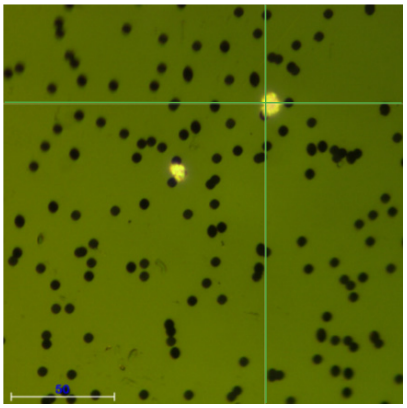


Figure 5: **(A)** Compound A Polymorph 1 particle identified by automatically scanning the filter round. **(B)** The Raman spectrum of the Compound A Polymorph 1 particle (blue) was matched to the custom reference spectrum for Compound A Polymorph 1 (green) with a rank of 952/1,000.

A



B

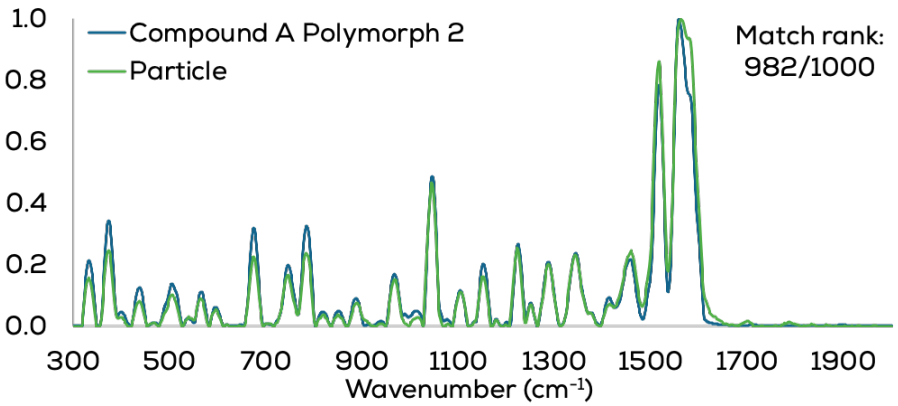


Figure 6: **(A)** Compound A Polymorph 2 particle identified by automatically scanning the filter round. **(B)** The Raman spectrum of the Compound A Polymorph 2 particle (blue) was matched to the custom reference spectrum for Compound A Polymorph 2 (green) with a rank of 982/1,000.

Conclusion

Hound provides a full count and size distribution of particles with the additional capability to perform Raman spectroscopy to identify the composition of particles. This combination creates a powerful tool for distinguishing polymorphs. Particle composition along with particle count and size provides the user detailed information on the ratio and distribution of polymorphs in each sample, which can be critical to evaluating the purity or stability of small molecule formulations.

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