2

Real-time Pharmaceutical Quality Analysis and Control using Handheld Raman Spectroscopy

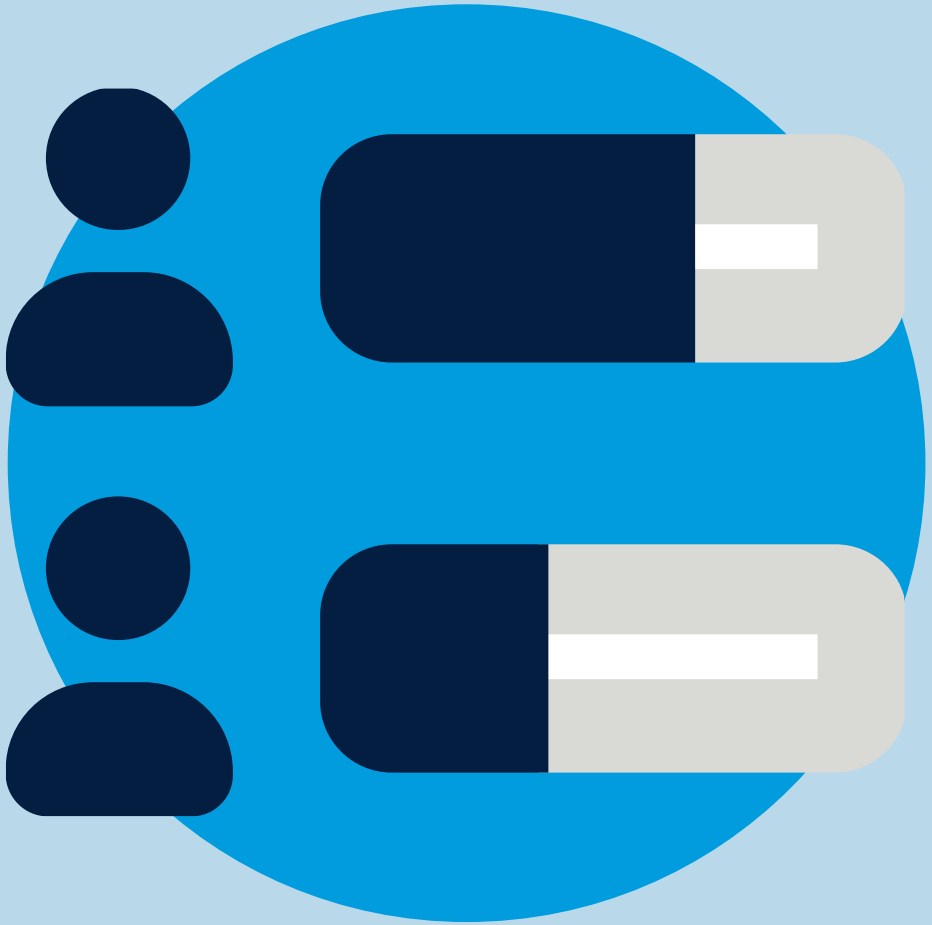
presented by Suzanne Schreyer, PhD

You will learn:

1. Fundamentals of Raman spectroscopy
2. Significance of handheld Raman
3. Applications for handheld Raman in pharma
4. Examples of handheld Raman in the pharma workflow
5. Case study: polymorph identification
6. Summary

Polling Question

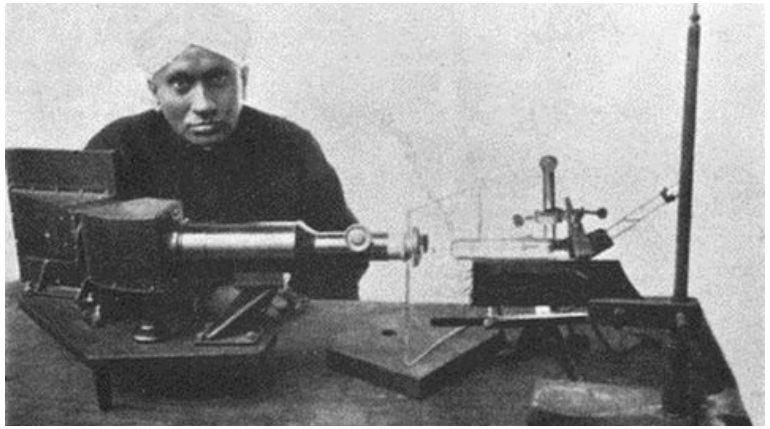
#1



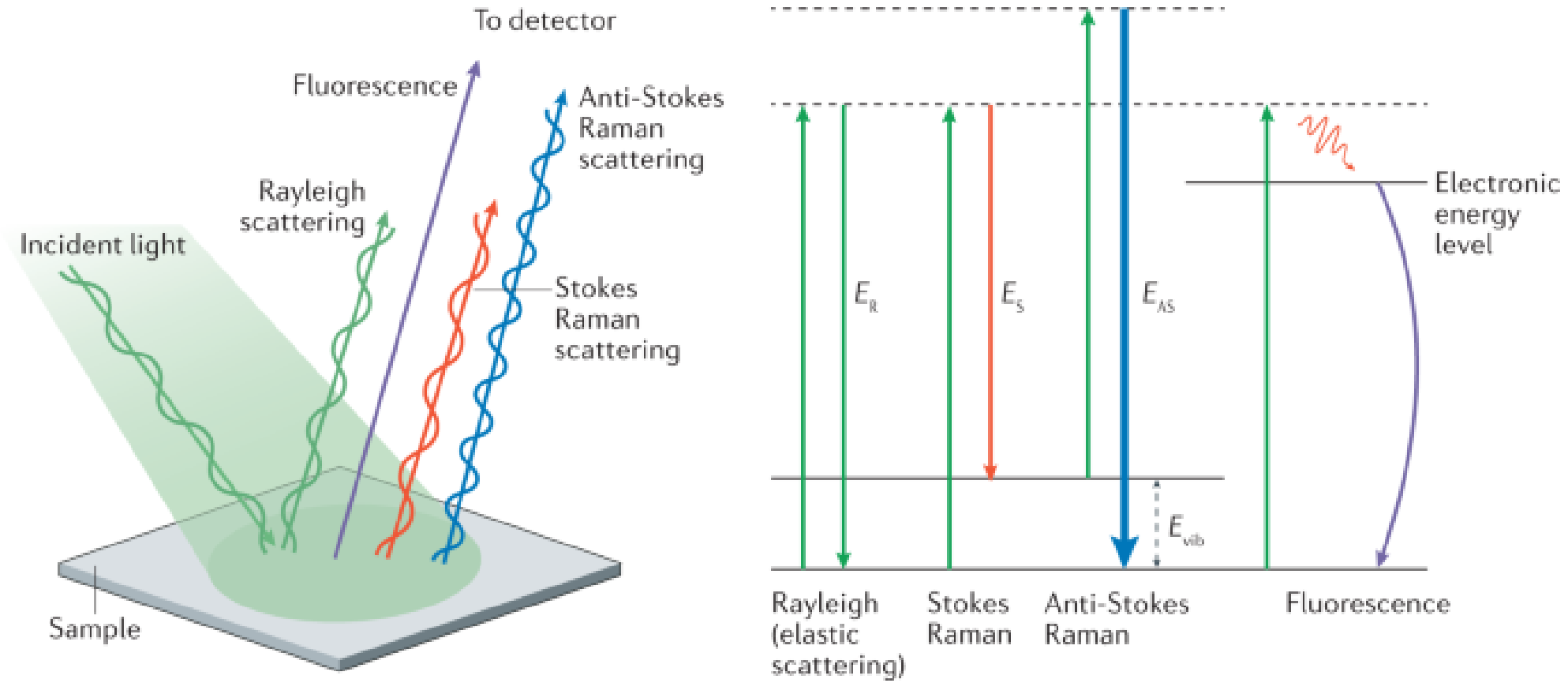
1. Fundamentals of Raman Spectroscopy

What is Raman Spectroscopy?

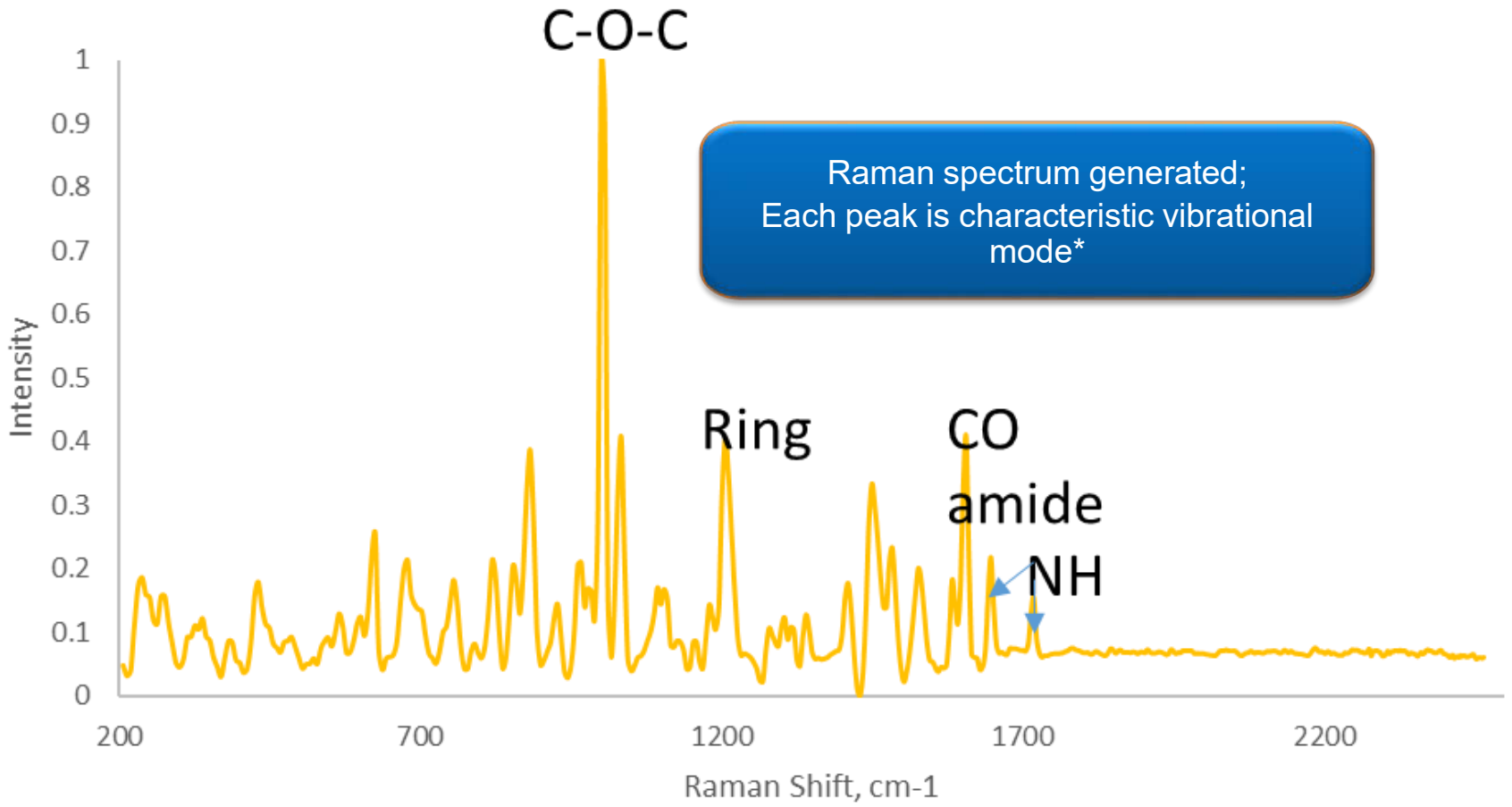
- Observed by C.V. Raman in 1928
- A Raman spectrum is a result of the inelastic scattering of light from a molecule
- Each molecule has a unique characteristic spectrum



What is Raman Spectroscopy?



What is Raman Spectroscopy?



Raman spectrum generated;
Each peak is characteristic vibrational mode*

=

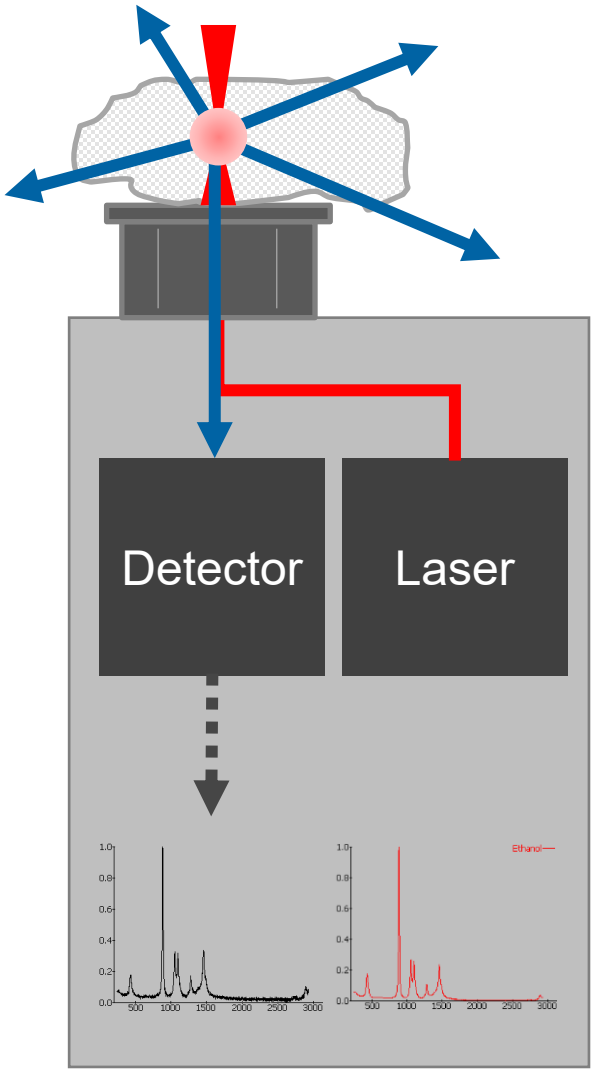
*as long as it's Raman-active

Benefits of Raman Spectroscopy

- Requires little to no sample preparation
- Nondestructive
- Can scan through some container types (i.e. plastic bags, glass bottles)
- Ability to scan: solids, liquids, powders, pastes, gels




2. Significance of Handheld Raman



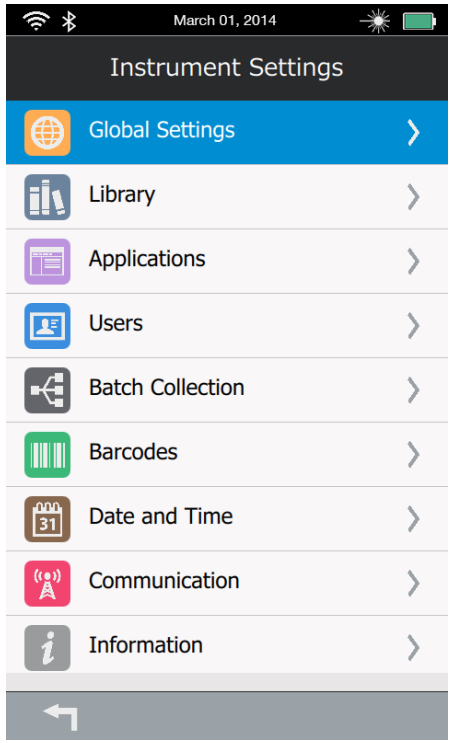
How Handheld Raman Works

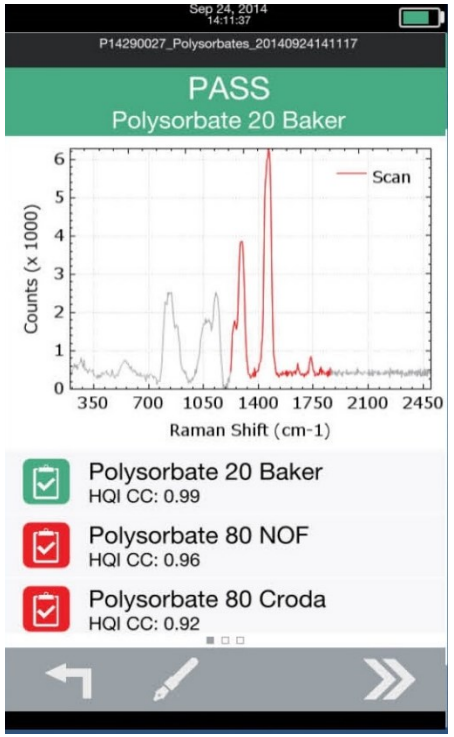
- Laser energy at a fixed single wavelength interacts with molecules in a material
- Light is scattered because of the interaction
- A detector collects some of the scattered light (**signal**)
- A computer processes the data and generates a Raman **spectrum** that is **unique** to the material(s) scanned.
- The collected spectrum is compared to a **library of known compounds**, and if a match is found…

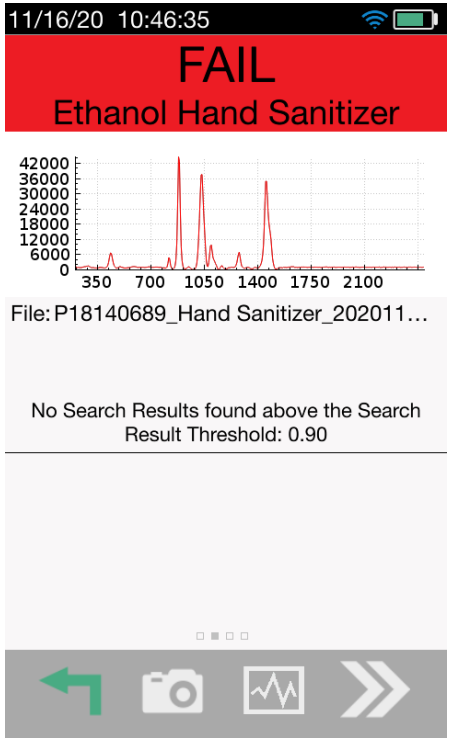
Identification!

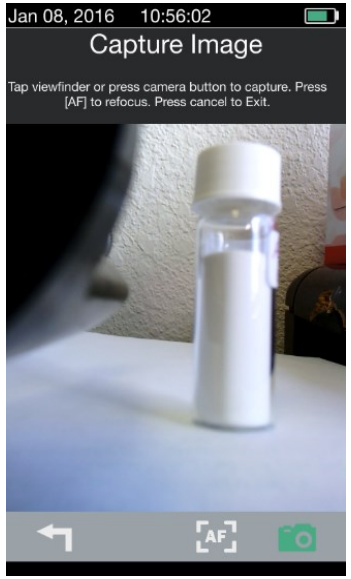
- Rigaku handheld Raman – Progeny 1064 nm
  - Results in seconds
  - Easy for the non-scientist to operate
  - Single-hand operation
  - Sealed system (IP-68) for warehouse use
  - Full control of analysis parameters
  - On-board barcode scanner / camera

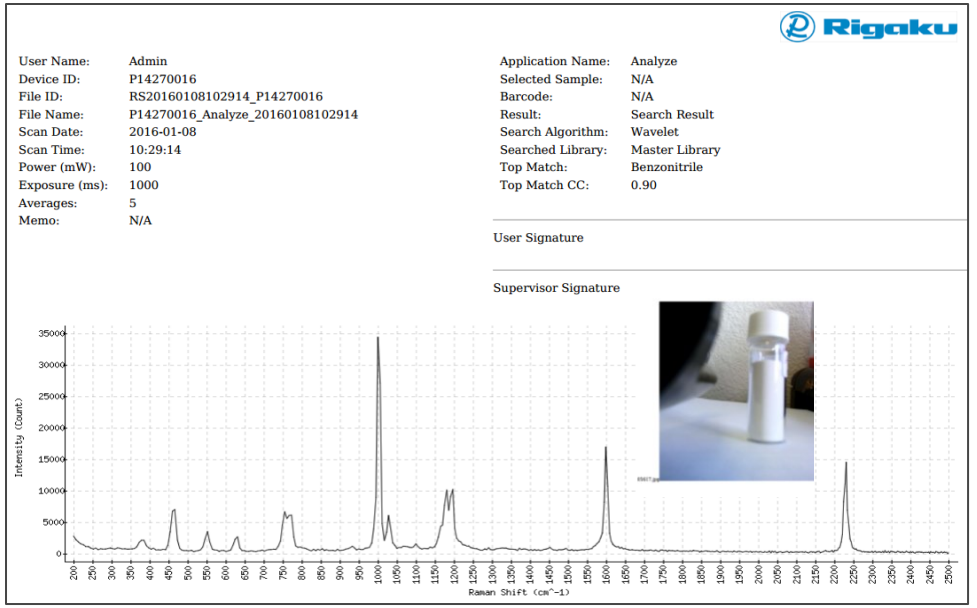
Progeny handheld Raman screen examples

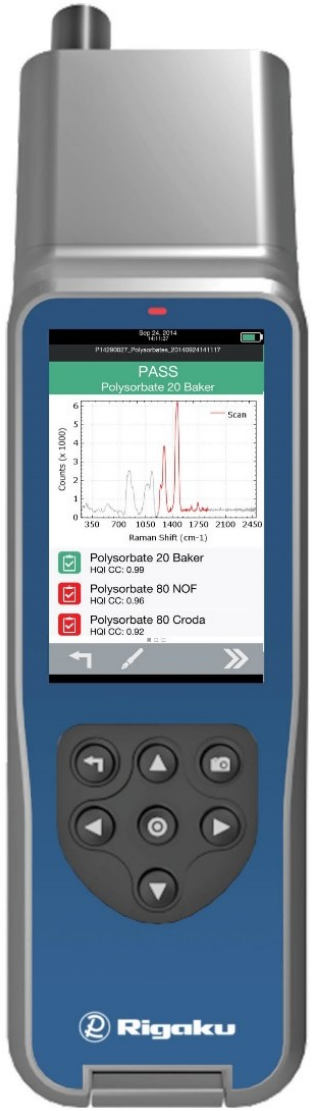












Regulatory acceptance of Handheld Raman

- Raman spectroscopy monographs compliance:
  - USP 1858 / USP 858 – replacement for USP 1120
  - European Pharmacopoeia (EP) Supplement 8.7, Chapter 2.2.48
  - Japanese Pharmacopoeia (JP) General Chapter 2.26 Raman Spectroscopy
- Compliance with U.S. FDA 21 CFR Part 11

Each instrument undergoes testing to ensure compliance for appropriate handheld standards (qualitative measurements)

Questions?

3. Applications for handheld Raman in pharma

Applications for Handheld Raman

Raw Material Identification:

- Active pharmaceutical ingredients (API)
- Excipients
- Nutraceuticals
- Pre-formulated materials and packaging

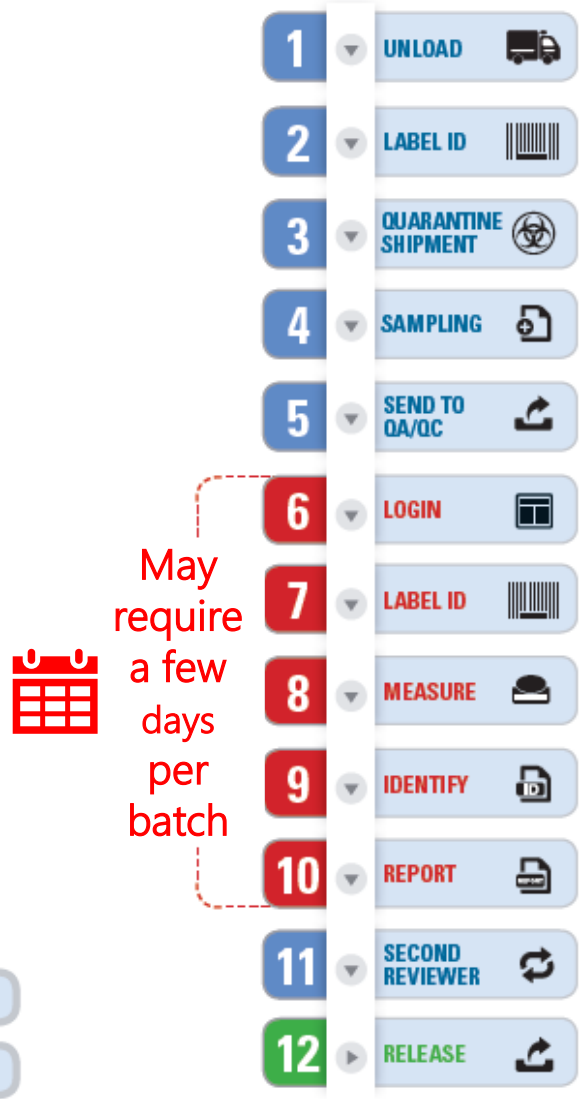
Verification:

- Pre- and post-clinical trial materials
- Chemicals and solvents
- Cell culture media

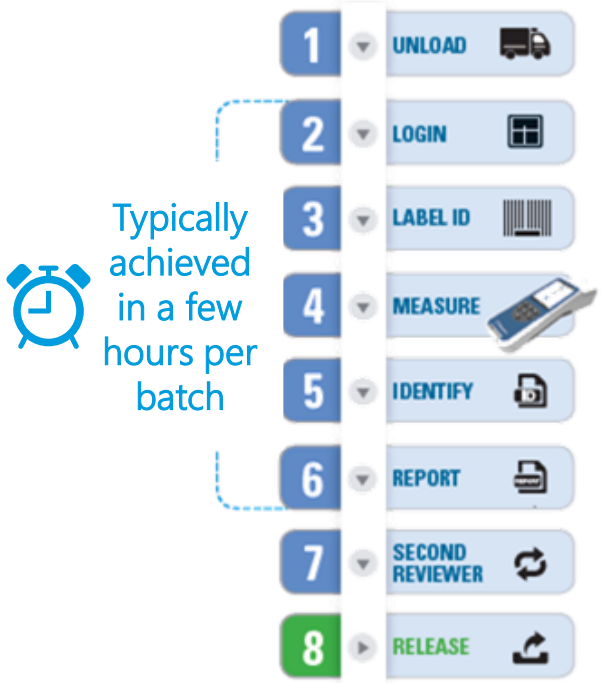
Authentication:

- Finished products
- Anti-counterfeit/brand security

TRADITIONAL RMID WORKFLOW:



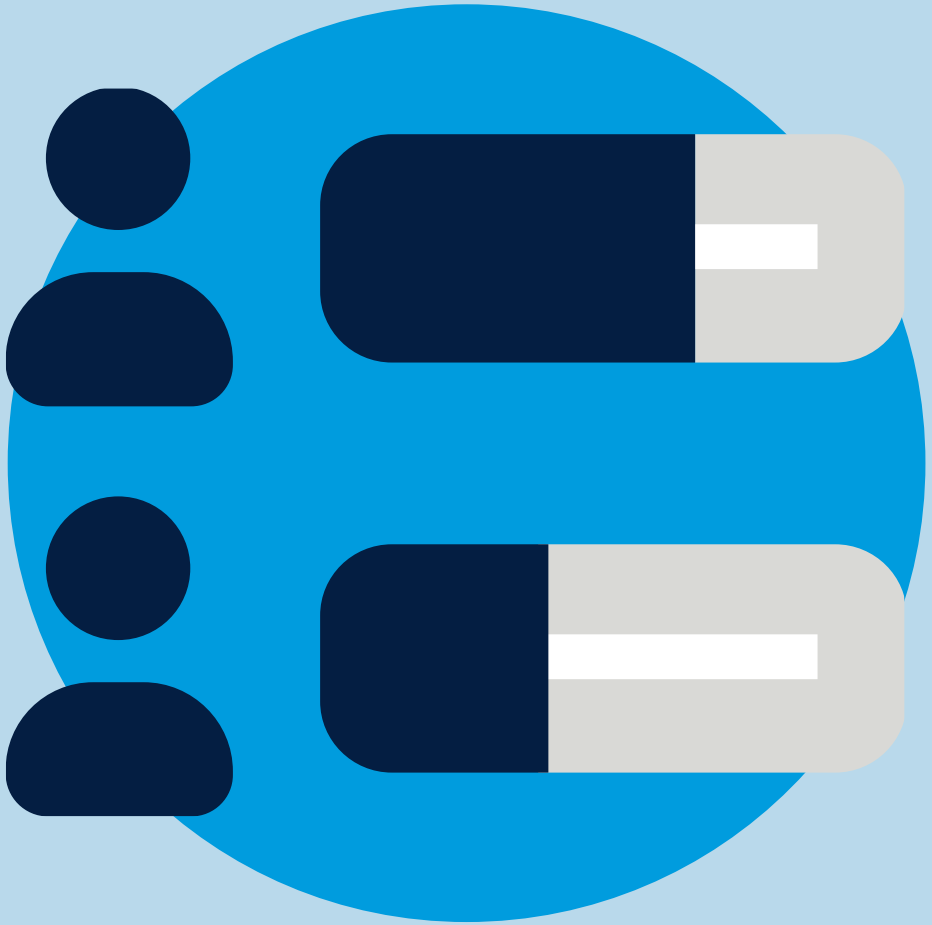
HANDHELD RAMAN WORKFLOW:



- ✓ No risk of contamination
- ✓ No waiting time in case of FAIL
- ✓ Less movement
- ✓ Increase inspection rates toward 100% ID
- ✓ Lower cost per analysis

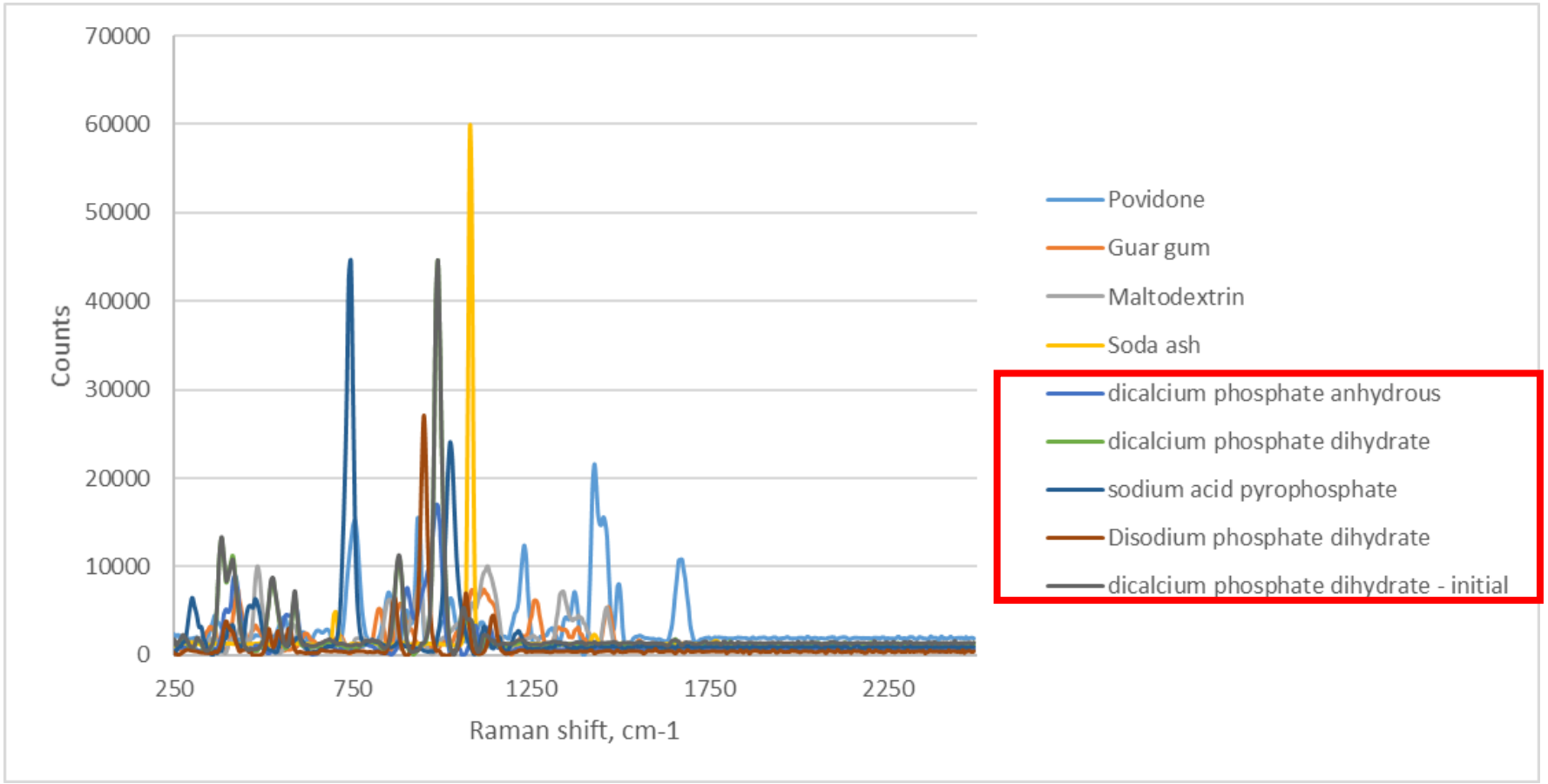
Polling Question

#2



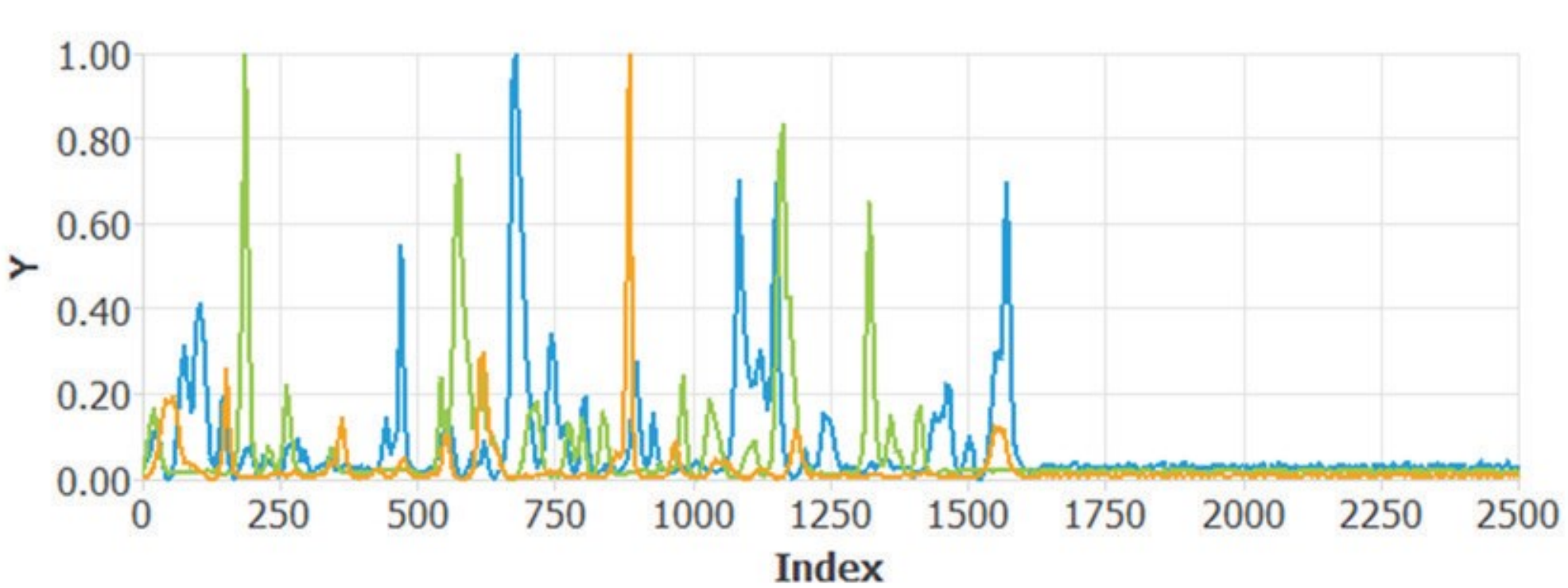
4. Examples of handheld Raman in the pharma workflow

Example #1: QA/QC



Example #2: Process Monitoring

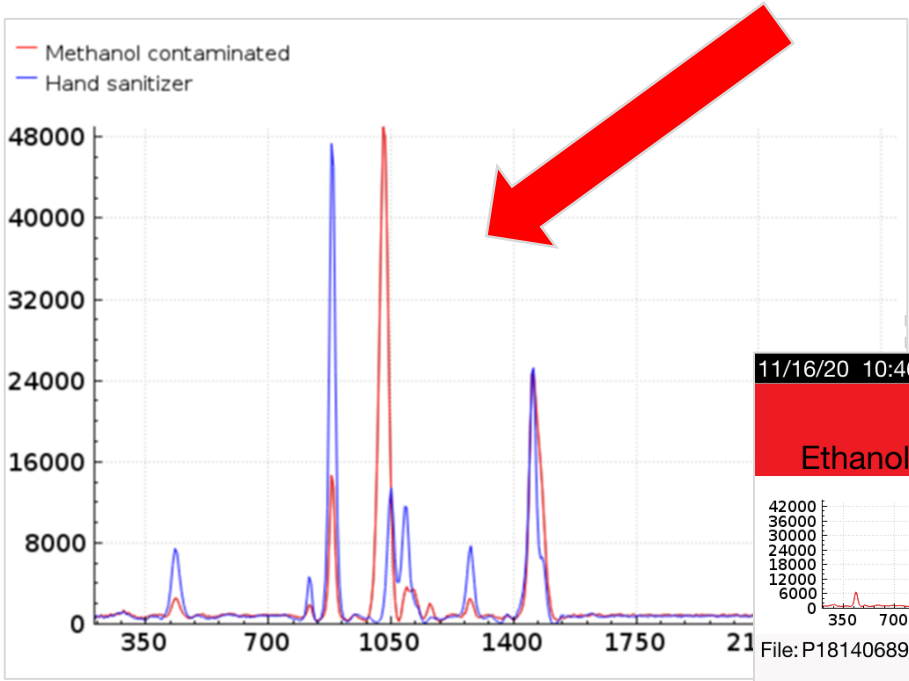
Epichlorohydrin + Methoxy phenol → Ritonavir intermediate

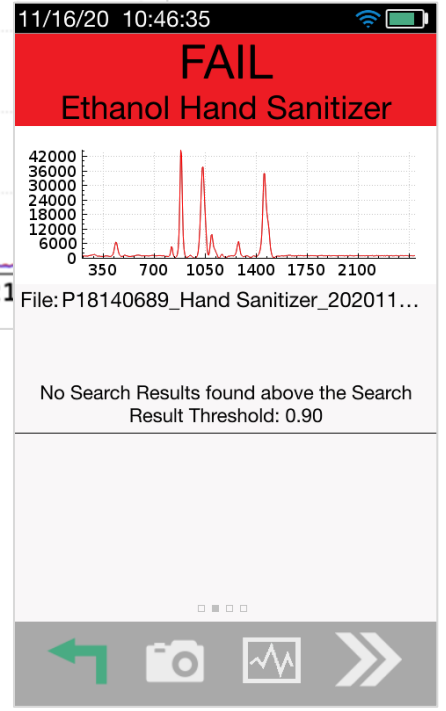


Example #3: Authentication

- Contamination of ethanol-based hand sanitizers
  - Methanol is a health hazard
  - Reduces efficacy of product
- Scan finished product to verify material
  - Ensures efficacy and safety

Shift in peak





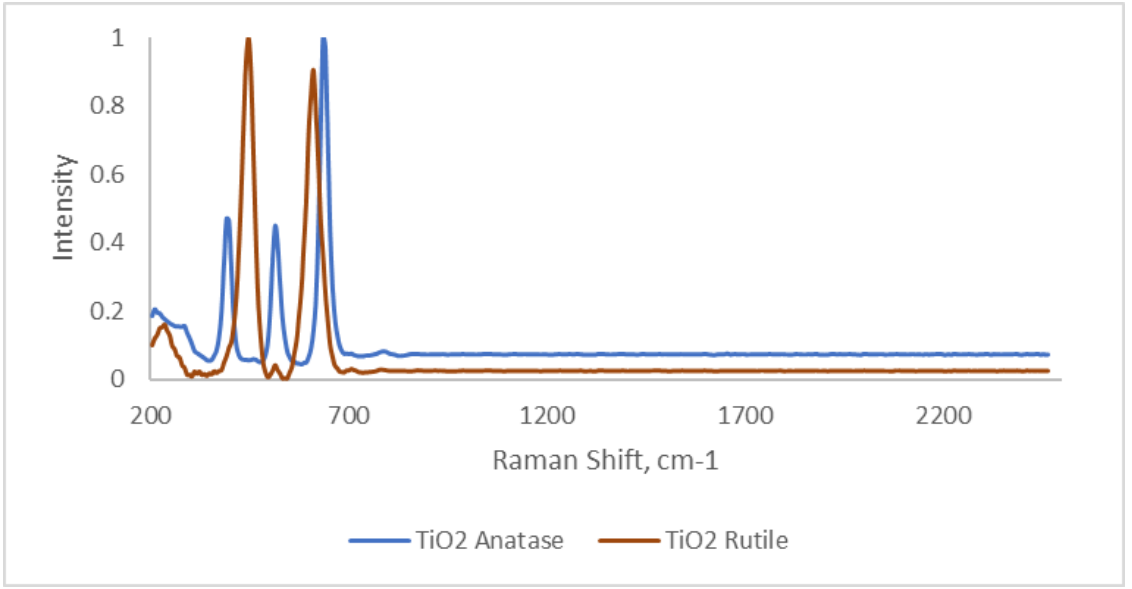
5. Case Study: Polymorph Identification

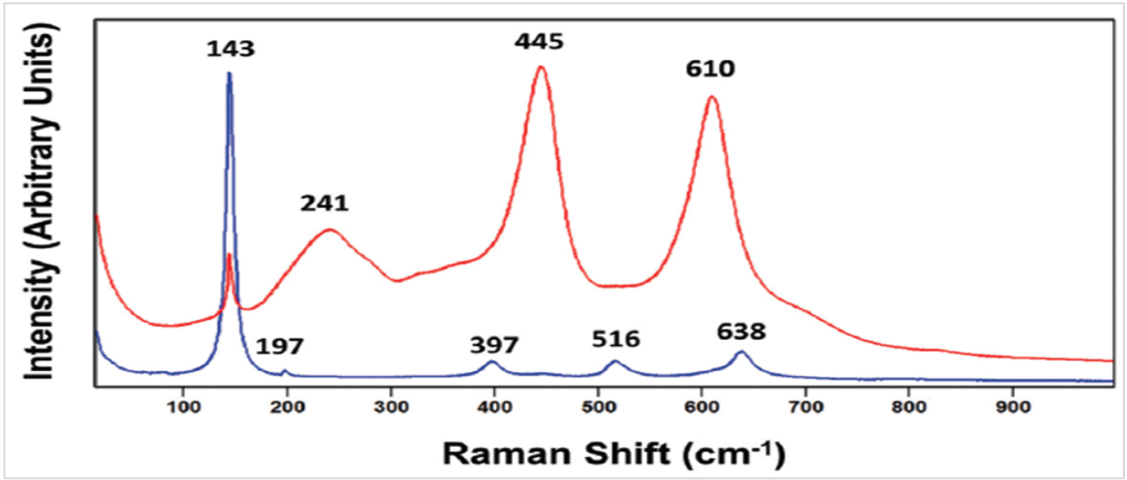
Polymorphs in Pharma Products

- Polymorphism – chemicals can exist in different crystalline forms
- Polymorphs impact physical properties, such as:
  - Dissolution rate
  - Bioavailability
  - Stability
- Results in Raman spectral changes
  - Raman is sensitive to packing and crystallinity changes
- Pharma SOPs require effects of polymorphism when developing specifications for a new drug substances

Coating Materials – Titanium Oxides

- Titanium oxide polymorphs exist in 3 common forms
- 2 are the most common in pharma:
  - Rutile (red)
    - Peaks at 445, 610 cm-1
  - Anatase (blue)
    - Peaks at 397, 516, 638 cm-1

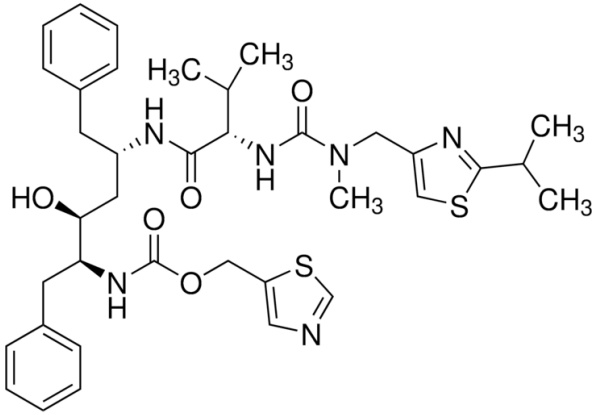


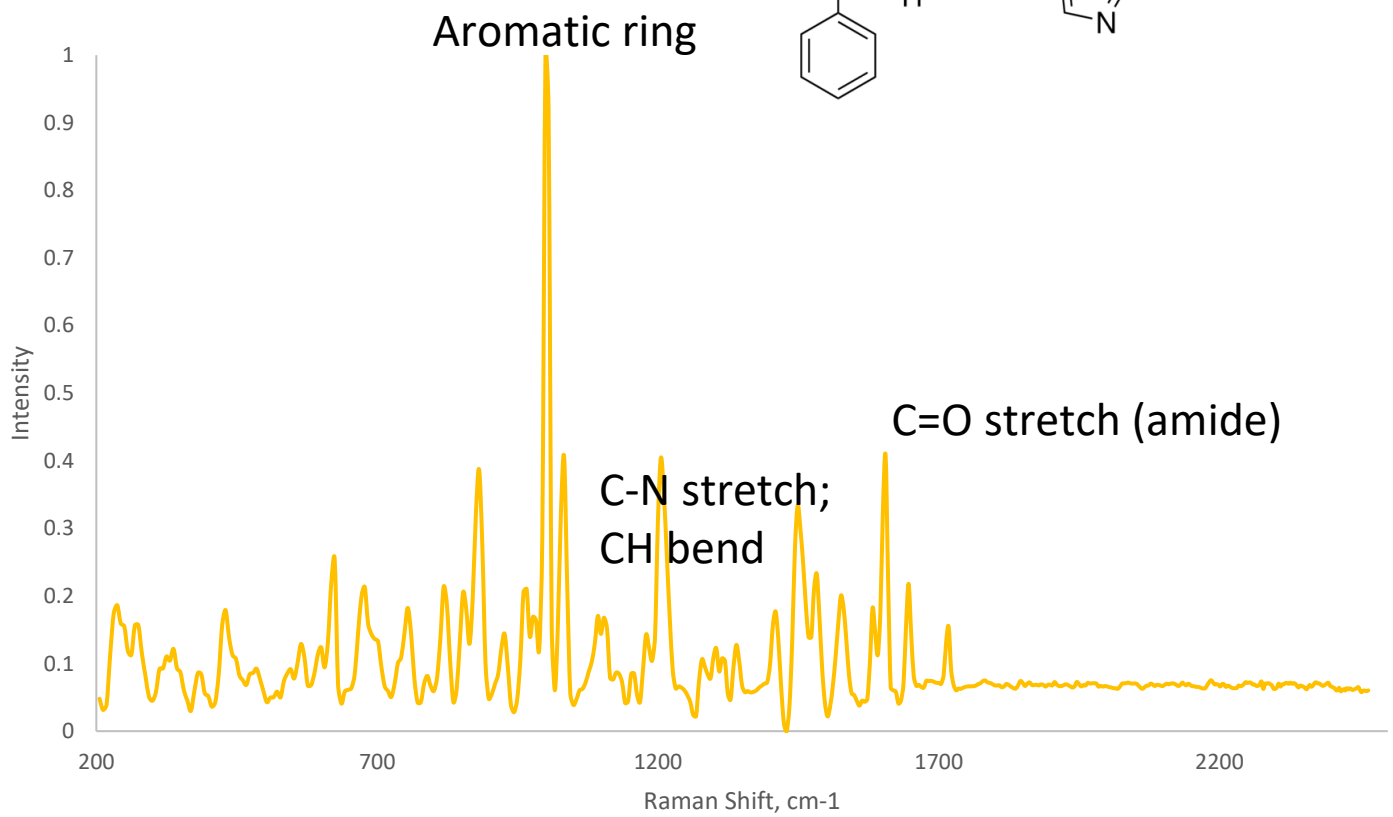


David Tuschel, Spectroscopy, 34 (3), pp10-21

Active ingredients - Ritonavir

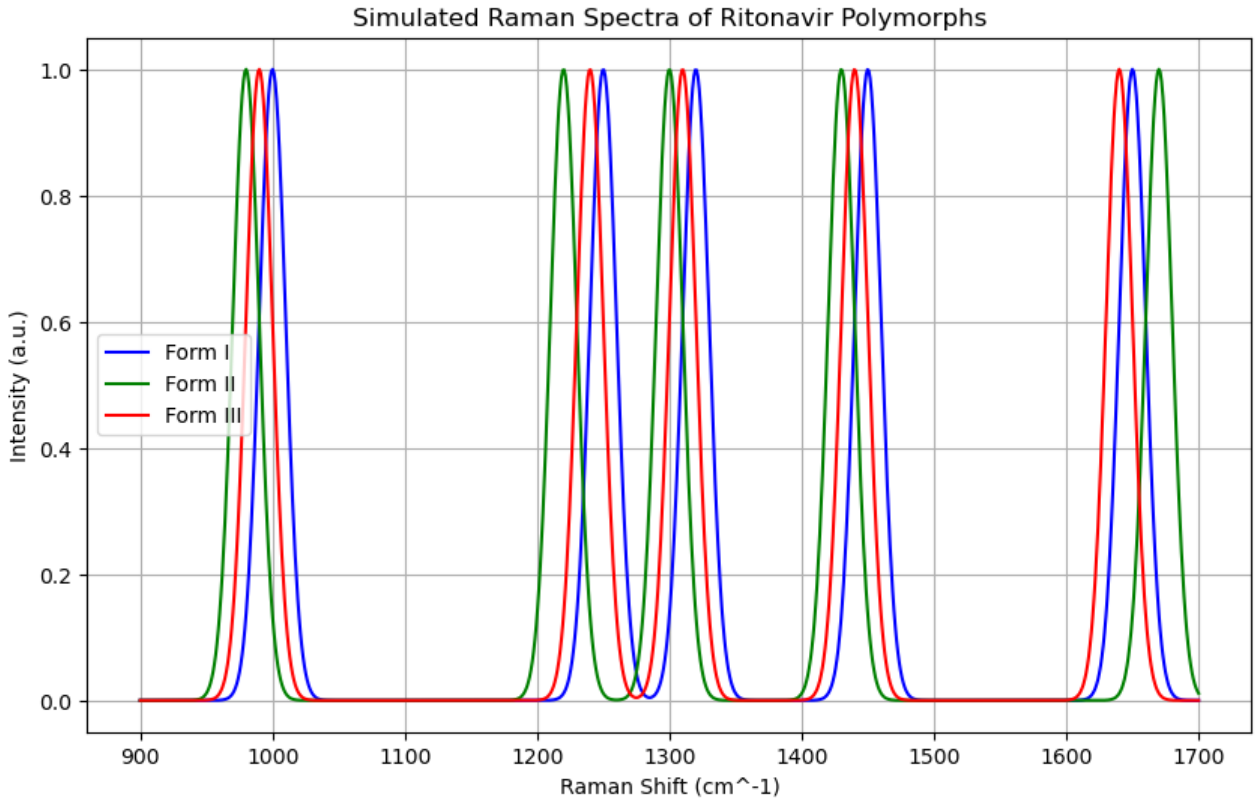
- Antiretroviral used to treat HIV-1
- 3 common polymorphic forms
  - Affects solubility
  - Affects bioavailability
- Withdrawn due to different polymorph form
- Polymorph forms are easily visible on the Raman spectra





Active ingredients - Ritonavir

- Polymorph forms and Raman spectra
  - Form I: Blue (marketable form)
  - Form II: Green
  - Form III: Red
- Correlation between spectra computed
  - Set cutoff threshold
  - Easy to selectively separate the forms



OpenAI. (2025, June 10). *Simulated Raman spectra of Ritonavir polymorphs (Forms I, II, and III)* [AI-generated figure]. Microsoft Copilot. https://copilot.microsoft.com

[Pharma Focus Europe: "Ritonavir: How the Discovery of a New Polymorph Changed Drug Development Forever"]

Pharmaceutical Research, Vol. 18, No. 6, 2001 — *Research Paper*

Ritonavir: An Extraordinary Example of Conformational Polymorphism

John Bauer,[1,2] Stephen Spanton,[1] Rodger Henry,[1] John Quick,[1] Walter Dziki,[1] William Porter,[1] and John Morris[1]

Received February 15, 2001; accepted March 10, 2001

Purpose. In the summer of 1998, Norvir semi-solid capsules supplies were threatened as a result of a new much less soluble crystal form of ritonavir. This report provides characterization of the two polymorphs and the structures and hydrogen bonding network for each form.
Methods. Ritonavir polymorphism was investigated using solid state spectroscopy and microscopy techniques including solid state NMR, Near Infrared Spectroscopy, powder X-ray Diffraction and Single crystal X-ray. A sensitive seed detection test was developed.
Results. Ritonavir polymorphs were thoroughly characterized and the structures determined. An unusual conformation was found for form II that results in a strong hydrogen bonding network A possible mechanism for heterogeneous nucleation of form II was investigated.
Conclusions. Ritonavir was found to exhibit conformational polymorphism with two unique crystal lattices having significantly different solubility properties. Although the polymorph (form II) corresponding to the "cis" conformation is a more stable packing arrangement, nucleation, even in the presence of form II seeds, is energetically unfavored except in highly supersaturated solutions. The coincidence of a highly supersaturated solution and a probable heterogeneous nucleation by a degradation product resulted in the sudden appearance of the more stable form II polymorph.

KEY WORDS: polymorphism; crystal forms; ritonavir; Norvir; carbamate; AIDS drug.

INTRODUCTION

Polymorphism is the ability for a compound to exist in more than one crystal form with different unit cell parameters. These individual crystal forms or polymorphs can exhibit differences in physical properties reflective of the crystal lattice. For example, the solubility of different polymorphs of the same compound reflect the differences in free energy between their respective crystalline states, which are different for each polymorph, and the solvated state. Thus a large range in equilibrium solubilities can exist for the various crystal forms of a compound. Differences in solubility between crystal forms of a pharmaceutical can lead to differences in maceuticals as in the case of the antiviral compound ritonavir. Ritonavir (I), [5S-(5R*,8R*,10R*,11R*)]-10-Hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid, 5-thiazolylmethyl ester, is a novel protease inhibitor (12–14) marketed in 1996 as Norvir oral liquid and Norvir semi-solid capsules for treatment of Acquired Immunodeficiency Syndrome (AIDS). Since ritonavir is not bioavailable from the solid state, both formulations contained ritonavir in ethanol/water based solutions. Therefore no crystal form control was required (15). The ICH (International Committee on Harmonization) guideline states "For a drug product that is a solution, there is little scientific rationale for polymorph control"

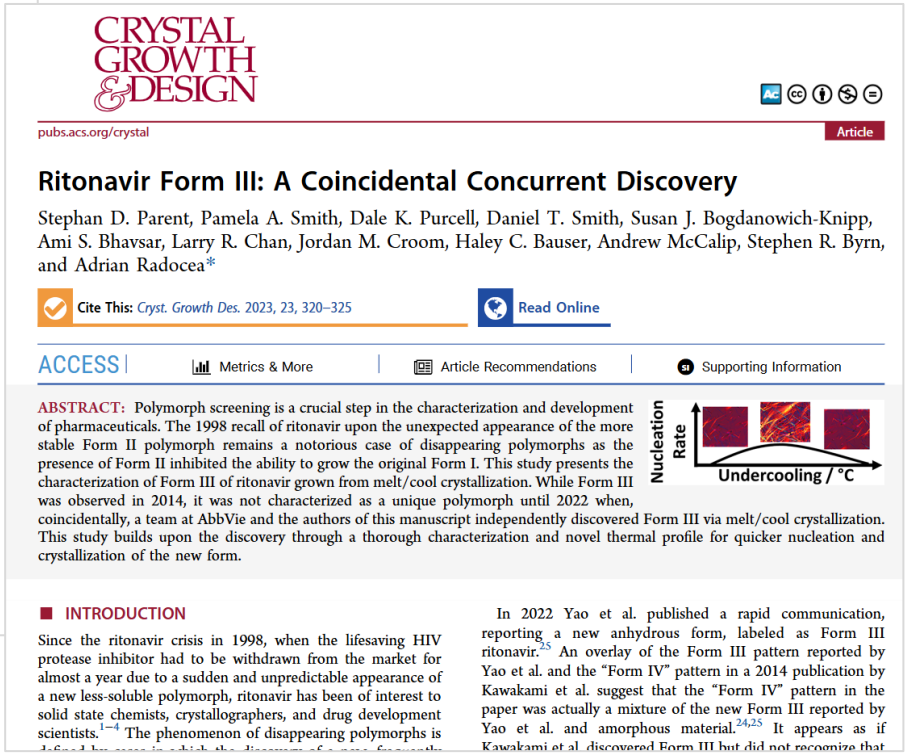
Only one crystal form of ritonavir was identified during development of the compound and 240 lots of Norvir capsules were produced with no stability problems.

In mid-1998, however, several lots of capsules failed the dissolution requirement and when capsule contents were examined using microscopy and X-ray powder diffraction, a new polymorph was identified that had greatly reduced solubility compared to the original crystal form. This new form, referred to as form II, is an example of conformational polymorphism, which occurs when different conformational isomers of a compound crystallize as distinct polymorphs (16). Within weeks this new polymorph began to appear throughout both the bulk drug and formulation areas. Since the manufacture of Norvir semi-solid capsules formulation involved the preparation of a hydroalcoholic solution of ritonavir which although not saturated with respect to form I was 400% supersaturated with respect to form II, the sudden appearance and dominance of this dramatically less soluble crystal form made this formulation unmanufacturable. Additionally Norvir oral solution could no longer be stored at 2–8°C without the risk of crystallization. These factors combined to limit inventory and seriously threatened the supply of this life saving treatment for AIDS. It was necessary to immediately reformulate Norvir. Form II of ritonavir was found to be both unusually stable and at the same time unusually difficult to crystallize. This report provides characterization of the two polymorphs and explanation of the unusual properties of form II.

EXPERIMENTS

Reagents

[Pharmaceutical Research: "Ritonavir: An Extraordinary Example of Conformational Polymorphism"]



CRYSTAL GROWTH & DESIGN

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Article

Ritonavir Form III: A Coincidental Concurrent Discovery

Stephan D. Parent, Pamela A. Smith, Dale K. Purcell, Daniel T. Smith, Susan J. Bogdanowich-Knipp, Ami S. Bhavsar, Larry R. Chan, Jordan M. Croom, Haley C. Bauser, Andrew McCalip, Stephen R. Byrn, and Adrian Radocea*

Cite This: *Cryst. Growth Des.* 2023, 23, 320–325

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ABSTRACT: Polymorph screening is a crucial step in the characterization and development of pharmaceuticals. The 1998 recall of ritonavir upon the unexpected appearance of the more stable Form II polymorph remains a notorious case of disappearing polymorphs as the presence of Form II inhibited the ability to grow the original Form I. This study presents the characterization of Form III of ritonavir grown from melt/cool crystallization. While Form III was observed in 2014, it was not characterized as a unique polymorph until 2022 when, coincidentally, a team at AbbVie and the authors of this manuscript independently discovered Form III via melt/cool crystallization. This study builds upon the discovery through a thorough characterization and novel thermal profile for quicker nucleation and crystallization of the new form.

INTRODUCTION

Since the ritonavir crisis in 1998, when the lifesaving HIV protease inhibitor had to be withdrawn from the market for almost a year due to a sudden and unpredictable appearance of a new less-soluble polymorph, ritonavir has been of interest to solid state chemists, crystallographers, and drug development scientists.[1−4] The phenomenon of disappearing polymorphs is

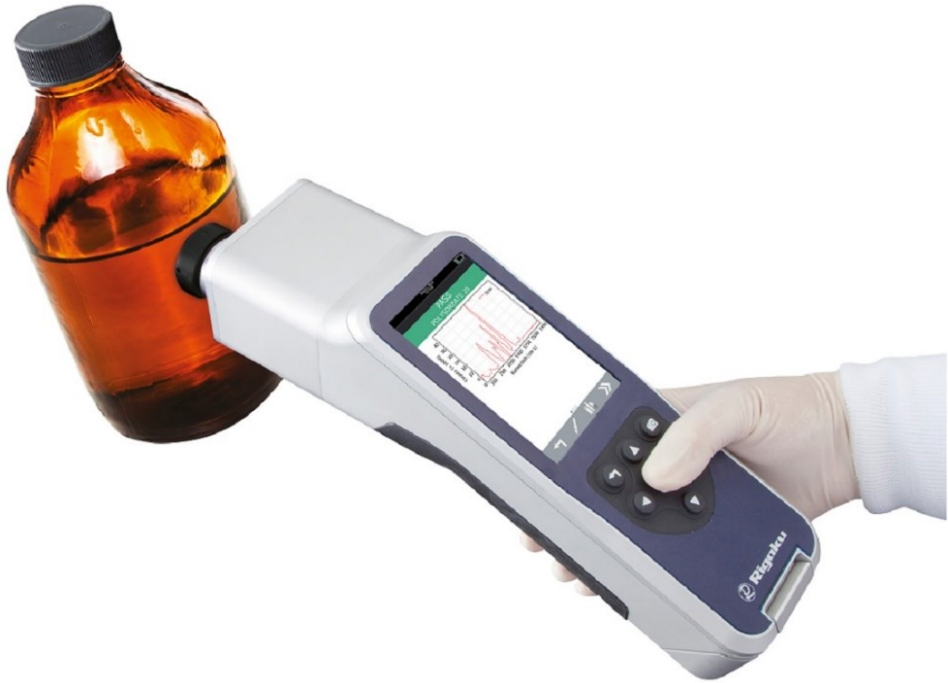
In 2022 Yao et al. published a rapid communication, reporting a new anhydrous form, labeled as Form III ritonavir.[25] An overlay of the Form III pattern reported by Yao et al. and the "Form IV" pattern in a 2014 publication by Kawakami et al. suggest that the "Form IV" pattern in the paper was actually a mixture of the new Form III reported by Yao et al. and amorphous material.[24,25] It appears as if Kawakami et al. discovered Form III but did not recognize that

[Crystal Growth & Design: "Ritonavir Form III: A Coincidental Concurrent Discovery"]

6. Summary

Handheld Raman in Pharma

- Can provide 100% inspection of incoming materials
- Beyond RMID – integrated into pharmaceutical workflow
  - Process monitor a reaction
  - Verification of finished formulations
  - Selectivity checks in QA/QC
  - Counterfeit / Adulteration detection



Questions?

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