An Executive Summary

Workflow Breakthroughs That are Improving Data Quality and Efficiency

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New technologies are changing pharmaceutical analysis by offering risk reduction and improved productivity while retaining data quality.

Introduction

Rapid results, coupled with reliable data integrity, are critical for routine analysis in pharmaceutical manufacturing and quality control. New breakthrough technologies are making an impact by dramatically reducing the risks involved in routine analysis while also decreasing the test time from hours or days to minutes. Advances in Raman spectroscopy improve the speed and efficiency of pharmaceutical testing, including through-container raw material identification (RMID), non-destructive tablet or capsule polymorph analysis, and content uniformity (CU) in batch release. A new molecular spectroscopy technique, Laser Direct Infrared Spectroscopy, is making sensitive quantitation of crystallinity and high-quality surface imaging of entire tablets possible in just a few minutes. In addition, a new ultraviolet–visible spectroscopy (UV-Vis) technology allows simultaneous measurement of standards and unknown samples across eight cuvette positions in a single experiment. These developments deliver both productivity gains and utmost data quality.

Accelerating Drug Discovery and Minimizing Error in Pharmaceutical Quality Control

Validation and qualification in pharmaceutical quality assurance/quality control (QA/QC) can be challenging and time consuming. The number of steps and the complexity of required protocols are laborious and introduce the potential for errors. In addition, analyzing samples and controls at different times presents the risk for variability as a result of operator error, accidents, and changing environmental factors, which can significantly reduce data quality.

In contrast, simultaneous measurement of samples and controls remove unnecessary steps and streamline analyses. A new UV-Vis spectrophotometer from Agilent, the Cary 3500, is capable of running up to four different experiments at once, enabling concurrent calibrations and sample measurements, accelerating workflows, and eliminating the potential variability of the measurement process. Optimal data quality and integrity are achieved as the instrument generates a calibration curve and reports sample concentration in a single step that takes less than five seconds.

The Cary 3500 Multizone UV-Vis allows up to four different temperature zones to be configured. Multiple cells at multiple temperatures can be measured simultaneously and the water-less temperature control can ramp at speeds up to 30 °C/min. Unlike traditional instruments that experience errors ranging from misaligned optics and missed data

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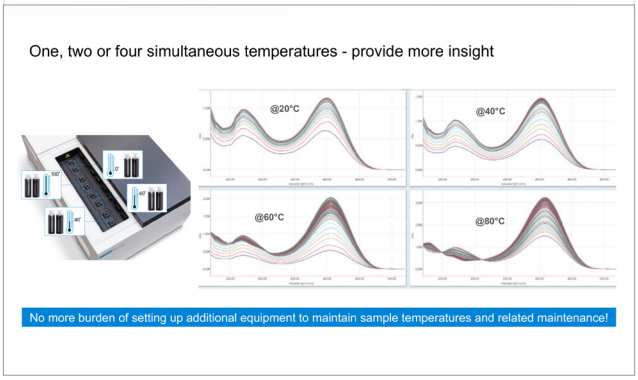
Figure 1: Quickly understand the impact of temperature on chemical reactions.



because of multi-cell changer movement, the Cary 3500's permanent optical alignment and lack of moving parts avoid those pitfalls. Additionally, the 250 points-per-second data collection rate, combined with the superfast Xenon Flash lamp, ensures that critical information gets captured. Its superior absorbance range eliminates the need for dilutions as well. The space-saving modular design can be mated to five different interchangeable measurement modules to meet the specific needs of a laboratory.

The Cary UV Workstation software has security, ease-of-use, and compliance features built in, thereby relieving administrative and operational burdens and allowing users to focus on science. The software includes streamlined method setup as well as a "Help & Learning" center for new or infrequent users. The secure database that contains application files with audit trails is easy to read, search, and filter. Access is protected by security settings and can be automatically backed up. Data recovery and audit traceability prevent data loss even in the event of a power failure. Conveniently, audit trails can be electronically reviewed, and the review is then permanently associated with the file.

Ideally suited for pharmaceutical laboratories, the Cary 3500's liquid sampling applications include fundamental characterization, investigation of thermal denaturation of proteins, and QA/QC testing of finished products. The instrument can be used to rapidly characterize and quantify the kinetics of reactions. For example, the hydrolysis of p-nitrophenyl acetate (pNPA) to p-nitrophenol (PNP) can be monitored at four different temperatures simultaneously to facilitate the study of the dependence of reaction rate on temperature. Measuring at four temperatures concurrently permits the reactions to be observed under the exact same conditions at the exact same time, with the only variable being temperature. Using this technique, the optimum temperature for the reaction to proceed can be readily determined. **Figure 1** demonstrates the impact of temperature on the chemical reaction.

Another helpful function of the Cary 3500 for pharmaceutical QA/QC laboratories is determining the concentration of unknown samples. Standards and a sample of unknown concentration are measured simultaneously, and a calibration curve is automatically generated. The concentration of a sample can then be easily ascertained. Since the standards and sample are all measured at the exact same point in time, all experimental variables are removed.

Clarity and Speed in Chemical Imaging

Innovations in vibrational spectroscopy have often delivered better chemical information for pharmaceutical applications. The Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System is capable of analyzing chemical constituents of large surfaces exceptionally quickly. This enables pharmaceutical laboratories to study changes in tablets during formulation development, visualize drug distribution in process development, or investigate defects in products. Manufacturing process information such as scale up or stability issues may be obtained as well.

> "Statistically significant results are acquired with equal sensitivity for active pharmaceutical ingredients (APIs) and excipients, while report creation is simple and straightforward."

The instrument uses a quantum cascade laser (QCL) that covers a large part of the infrared fingerprint region. Using this high-brightness laser source, a large area can be scanned with different wavelengths by rastering at high speed across the sample. This enables imaging of the components of a tablet surface at relatively high resolution within minutes and at the highest resolution in as little as one hour.

An intuitive interface with easy method setup and automated sample analysis allows consistent, robust operation of the 8700 LDIR regardless of user skill levels. Statistically significant results are acquired with equal sensitivity for active pharmaceutical ingredients (APIs) and excipients, while report creation is simple and straightforward.

Figure 2: Tablet formulation imaging.

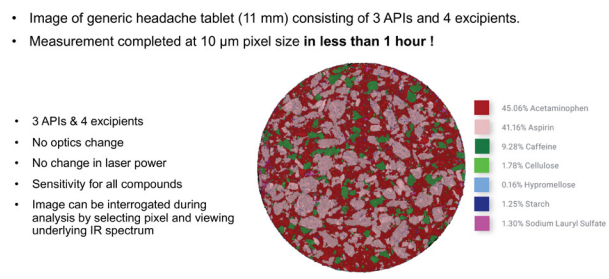
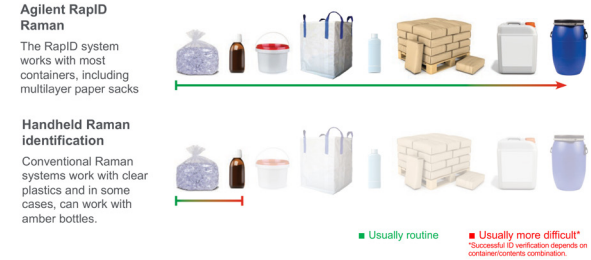


Figure 3: Container compatibility.



Two useful accessories are available that expand the applicability of the instrument. Integrated Attenuated Total Reflectance (ATR) increases the spatial resolution from a few microns down to 0.25 µm pixel size for an area of interest. Also, a sample planer may be used to shave samples in order to prepare a flat surface for imaging or to gain access to the inside of a sample.

Samples require no preparation and are directly inserted into the 8700 LDIR on a slide. Initially, a visual image of the sample(s) is produced, from which the user chooses an area of interest. The resolution is then chosen, as well as the type of components to be sought. It is also possible for the instrument to perform a pre-scan and find various sample components. The components will then be scanned for and identified according to specific wavelengths and chosen automatically as a function of the components being measured. A colored map is automatically created that illustrates the identity and location of the sample components. Automated sample analysis software can be used to produce a report.

An imaging map for an 11 mm generic headache tablet is shown in **Figure 2**. The three APIs and four excipients were detected with equal sensitivity with no need to change the optics or laser power. Note that the cellulose, hypromellose, starch, and sodium lauryl sulfate excipients would typically not produce a good Raman spectrum, but are responsive to LDIR. The experiment took less than an hour and measured pixel sizes of 10 µm. The IR spectrum for each pixel can be easily viewed if necessary. The simple, efficient workflow of the 8700 LDIR system enables higher productivity for faster, more accurate decision making compared to Raman-based methods.

Through Packaging Raw Material Identification

Good manufacturing practice (GMP) guidelines call for verification of raw materials before use in pharmaceutical manufacturing. This quality control requirement creates a burden on the pharmaceutical industry to analyze large numbers of materials as efficiently as possible. Rather than taking the time to obtain representative samples from each container to be tested, spatially offset Raman spectroscopy (SORS) can be employed using Agilent's RapID portable Raman spectrometer to 'see' through opaque and colored packaging. The technique effectively removes the container signal to reveal the signal from its contents so that they can be identified with high specificity. Unopened packaging remains intact, which saves time and avoids possible contamination. From a safety perspective, SORS is highly advantageous for the analysis of sterile or hazardous materials. RapID is the only instrument that can penetrate even highly opaque containers such as paper sacks.

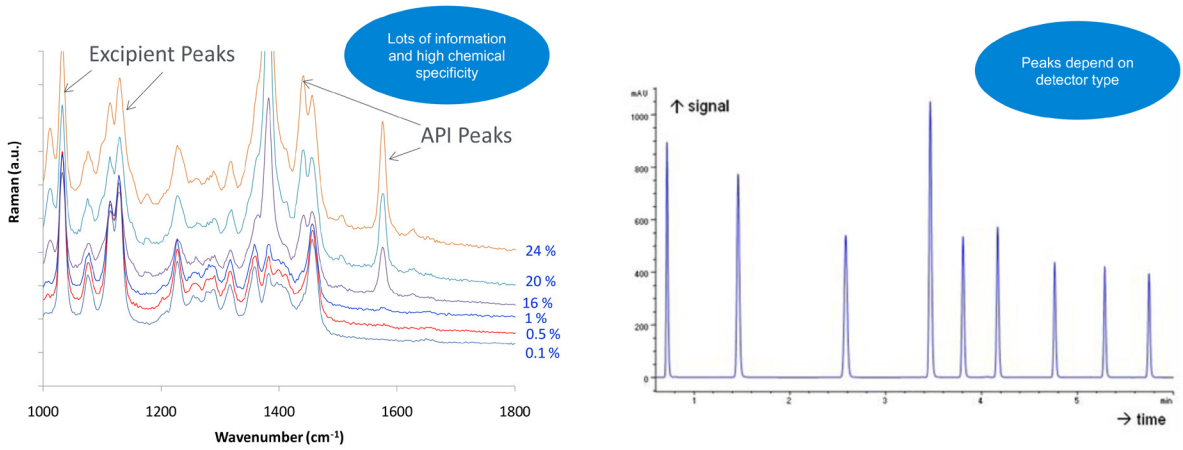
Because the instrument is used in the pharmaceutical industry, it is compliant with 21 *CFR* part 11. Production sites benefit from the instrument's seamless integration with existing lab and warehouse processes through a laboratory information management system.

RapID measurements done directly in the warehouse save considerable time compared to conventional measurements that involve many logistical operations. Moving containers from the warehouse to quarantine, then to the sampling booth for testing by gowned personnel, and then moving containers back to the warehouse is expensive and slow. RapID's unique SORS probe head is simply pushed against the container and a measurement is triggered that produces a result in seconds, enabling immediate release into production. Saving on handling and laboratory testing reduces overhead from hours or days to only minutes per batch. It is also extremely useful for easily testing the top, middle, and bottom contents of large containers for added certainty of supply. Furthermore, RapID can sample through a much wider variety of colored or fluorescent containers than traditional Raman, as shown in **Figure 3**.

Quantitative Content Uniformity and Polymorph Analysis

While the highly specific RapID system is ideal for identification of materials, quantitative applications are much more suited to transmission Raman spectroscopy (TRS). Agilent's TRS 100 is well suited to regulated oral solid dosage (OSD) form analysis such as content uniformity, assay and drug product ID. Simple to use with no sample

Figure 4: Information-rich, fast analysis.



TRS spectra captured in **1 second** on a TRS100
Single spectrum of the entire sample
Quantitation by multi-variate analysis

HPLC Chromatogram takes minutes
Peaks separated by elution time
Quantitation by peak area ratio with standard

preparation, the TRS 100 works with most dosage forms and the transmission geometry effectively samples all of the contents of a sample. OSDs are simply loaded into a tray and placed in the instrument where the computer takes over the analysis. Hundreds of intact tablets or capsules can be tested in just minutes. Changing between products is immediate and requires no modification of the instrument, unlike separation techniques, even if they require different methods. APIs and polymorphs can be quantified in a single, fast measurement.

TRS and high-performance liquid chromatography (HPLC) overlap in capability. Unlike LC, the TRS technique requires no consumables or solvents, generates no waste, and needs no physical changes between different types of samples. HPLC requires sample preparation, solvents, and repetitive runs of reference standards. HPLC results depend upon the column, materials, solvent, temperature and other factors. In TRS, a reference model is built based on known concentration variance of the excipients and API. Once the model is developed, it can be used repeatedly without reruns of reference materials.

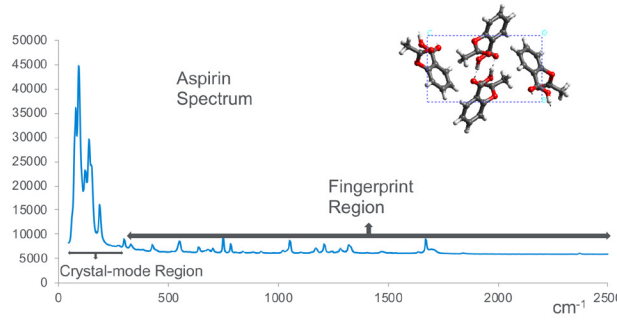
Figure 4 demonstrates how all of the needed information can be obtained with a one-second measurement on the TRS 100. Variances in API concentration between the six samples are readily visible at approximately 1600 and 1450 wavenumbers. The spectrum contains information about the relative concentration of API and excipients that can be modeled to create the percent of content, almost in real time. A single tablet takes approximately 20 seconds to analyze by TRS, so that 100 tablets only consumes about half an hour. This can lead to fast release times and high productivity. Moreover, the TRS 100's ease of use saves training time and allows users to focus on what matters.

One particular use of the TRS 100 involves looking at a formulated product that contains an amorphous API that re-crystallizes back to the crystalline state. **Figure 5** presents an example of how the sensitivity of whole object analysis can be used to quantify solid-state information inside a tablet. The intact tablet is placed into the transmission arm of the instrument and the crystallinity is quantified down to less than 1% limit of quantitation (LOQ) in just a few seconds. The technique is as sensitive as solid-state Nuclear Magnetic Resonance (ssNMR), yet it consumes a fraction of the time and cost. The information could also be gained by using X-ray diffraction (XRD) but only to approximately 5% LOQ and it would require sample preparation and several minutes of run time. Thus, TRS is especially useful for amorphous formulations in which relaxation back to the crystalline state is investigated.

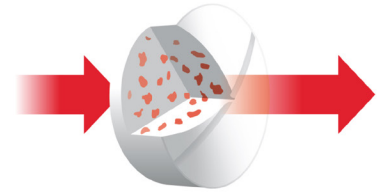
Conclusion

Recent workflow enhancements for Agilent's suite of molecular spectroscopy instruments have significantly improved accuracy and productivity for pharmaceutical analyses. The Cary 3500 UV-Vis Spectrophotometer has the capacity to rapidly measure eight samples/standards simultaneously, and can be configured with four different temperature zones. Its permanent optical alignment and no moving parts amplify the integrity of

Figure 5: Polymorph and solid state analysis.



Typical method for polymorph detection
XRD – destructive, ≈5% LOQ, minutes
TRS100 – non-destructive, <1% LOQ, seconds



TRS100 can measure solid-state form quantitatively

- Measure residual crystallinity in amorphous API drug product/substance
- Works with intact tablets and capsules
- Comparable to solid state NMR for sensitivity but in seconds and no prep

TRS combines chemical and crystal information in a single measurement

TRS quantifies the solid-state form, even if there is an uneven distribution of "hotspots"

No need for sample preparation – takes seconds

J.A. Griffen, A.W. Owen, J. Burley, V. Taresco, P. Matousek, Rapid quantification of low level polymorph content in a solid dose form using transmission Raman spectroscopy, J. Pharm. Biomed. Anal. 128 (2016) 35
M.C. Hennigan, A.G. Ryder, Quantitative polymorph contaminant analysis in tablets using Raman and near infra-red spectroscopies, J. Pharm. Biomed. Anal. 72 (2013) 163

the results. The instrument is ideal for many applications, including monitoring enzymatic reactions at four different temperatures, concurrently calibrating and determining sample concentration, and quantifying nucleotides and proteins with speed and accuracy.

The 8700 LDIR Chemical Imaging System brings clarity and unprecedented speed to chemical imaging of pharmaceutical samples as it generates fast high-resolution images. Automated and simple to operate, the instrument features a QCL light source for better performance and quality. The 8700 LDIR has proven to be a valuable tool for formulation development, drug distribution studies, stability investigations, and defect identification.

For quick and easy raw materials identification, Agilent has introduced the RapID hand-held Raman spectrometer. Using SORS technology, the instrument penetrates most types of packaging so that materials can be identified by simply touching the probe to the container. In addition to vastly accelerating GMP compliance, this eliminates sample handling, contamination, and the risk of exposure to hazardous materials.

The automated TRS 100 routinely quantifies solid-state sample constituents in seconds, providing fast content uniformity and assays in release testing. A single measurement can deliver quantitative chemical and polymorph information down to 1% LOQ with no sample preparation.

Agilent's suite of instruments has significantly reduced the complexity of pharmaceutical analysis, with streamlined workflows and simplified operation. The time savings for laboratories and manufacturers are enormous, as most of the protocols include automation and require no sample preparation or expert users. The workflows eliminate common errors associated with sampling and analysis, which strengthens confidence in the analytical results. As such, the workflow improvements have accelerated drug development and manufacture without compromising data quality.

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