Agilent Case Study

Interview: Evaluating an Innovative Analytical ID Testing Strategy for Oligonucleotides

Tell us about your company.

Vetter is a family-owned contract development and manufacturing organization (CDMO) headquartered in Ravensburg, Germany, with production facilities in Germany, Austria, and the United States. Currently employing more than 5,700 individuals worldwide, the company specializes in sterile injectables in a wide range of formats (syringes, cartridges, vials, and dual-chamber systems). The core services include clinical and commercial manufacturing, secondary packaging, and device assembly of parenteral medications.

Vetter has deep experience in supporting the production processes of their biotechnology and pharmaceutical customers through analytical services such as quality control (QC) testing for incoming goods, in-process and filled drug products, stability studies, and developmental analytics.

Learn more about Vetter at vetter-pharma.com.

What kind of work does your laboratory do?

The Analytical Science Laboratory (ASL) within Vetter's Development Service (VDS) is specialized in analytics for development and process characterization purposes in accordance with cGMP requirements. VDS supports the development of the customer's products, especially production or packaging processes.

The ASL provides analytical support for the development and implementation of production and filling processes, for example, by measuring residual moisture in developmental lyophilization studies, by determining protein concentration and stability in developmental mixing or pumping studies, or by supporting process development for the sanitization of clean rooms with our ASL "Center of Excellence H_2O_2 Determination".

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Vetter is one of the leading experts in the field of the filling of parenteral drugs, including ophthalmic products; therefore, a considerable part of our laboratory competencies covers subvisible particle analysis in our ASL "Center of Excellence Particulate Matter".

Additionally, we support the transfer of analytical methods from our customers, both to Vetter and within Vetter, through feasibility studies and laboratory qualification or procedure validation.

Finally, we support the company-wide implementation of new analytical procedures and technologies to constantly modernize and upgrade our analytical systems and provide state-of-the art technologies for new products.

Describe some of the challenges you encounter.

Oligonucleotides are an emerging class of biopharmaceuticals without a state-of-the-art filing strategy for product approval with regard to analytical techniques. As CDMO, Vetter requires smart analytical solutions to fulfill the needs of many customers with many different products. We must anticipate the most likely system or procedure candidates for the future needs of our customers for development and commercial production so we can provide the essential analytical data to assure patient safety and to fulfill regulatory requirements at reasonable time and effort.

Another challenge is that in cGMP environments, analytical system or procedure innovation or system upgrades are regulatorily demanding and often technically complex. An integrative approach that includes analytical technology specialists, quality assurance experts, and IT professionals is needed. Finding a solution that fulfills the data integrity requirements while enabling convenient use of the analytical system without losing necessary functions can be especially challenging. We strive to anticipate the future needs of our customers, and of Vetter itself, especially with respect to regulatory compliance, as early as possible.

How has the Agilent Cary 3500 UV-Vis spectrophotometer helped you overcome these challenges?

One of our current projects aims to optimize the analytics of oligonucleotide-based pharmaceuticals. For example, standard identity (ID) confirmation tests such as HPLC- or UPLC-based procedures typically fail to confirm the ID of such products.

There is a trend towards using melting temperature (Tm) in combination with intact mass measurement, combining sequence and mass to achieve unequivocal identification. Though different analytical techniques have been thoroughly reviewed, this Tm-based strategy has been discussed at expert conferences and is supported by many oligonucleotide-developing and -producing pharmaceutical companies.

For this approach, the Cary 3500 UV-Vis provides state-of-the-art technology. Temperature can be accurately set with the integrated Peltier element and controlled with the in-cuvette probes. The design of the multizone instrument with four cuvette pairs allows measuring samples and references in parallel, with up to four different temperature zones.

Why do you use UV-Vis as an oligonucleotide identification test?

A primary goal of any pharmaceutical manufacturing process is to ensure that every unit of drug product contains the designated pharmaceutical product of correct amount and quality. This is achieved by high-quality manufacturing processes and QC testing.

Current QC practices in ID testing include highly sophisticated technologies such as de novo sequence confirmation by MS/MS sequencing or failure sequence analysis. Especially for single-stranded oligonucleotides, intact mass measurement can be coupled with a second, sequence-sensitive technique such as MS-fragmentation pattern, Tm, or NMR analysis and compared with a fully characterized reference standard. For double-stranded oligonucleotides, ID testing may be even reduced to an intact mass measurement coupled with confirmation of the formed double-strand, if intact masses of the individual single strands are also measured before annealing.[1]

While there are other possibilities, we are convinced that a combination of measuring Tm and mass will be the standard analytical strategy for QC ID testing in the future. This is sound both scientifically and from a regulatory and business point of view; implementing UV-based Tm systems and procedures in QC laboratories is relatively easy and has a low cost of ownership.

Methods are less complex than other approaches such as MS/MS sequencing or NMR, and there is often skilled staff available that can quickly be trained on the new instruments and procedures instead of hiring highly trained experts.

These instruments can also perform standard UV-Vis methods, so they can be integrated into the existing instrument pool and will deliver a high degree of capacity utilization right from the beginning. At Vetter, we have successfully implemented this in the ASL as well as in the QC laboratory.

How did you use UV-Vis using the Cary 3500 into an oligonucleotide identification test?

To gain in-depth knowledge about the instrument and the Tm application, we performed several studies. An investigation of instrument performance using different parameter settings resulted in very robust performance. This work was discussed with expert audiences and is detailed in a recent white paper.[2–4]

In another study aimed at determining the sensitivity of Tm measurements for ID testing, we investigated the specificity of the procedure towards various oligonucleotide sequences. Model oligonucleotide sequences and variants were designed, synthesized, and analyzed; we found that even single-base exchange or single-nucleotide deletions or insertions led to significant changes in the measured Tm of the oligonucleotide duplexes. The generally high specificity of the method towards subtle changes in oligonucleotide sequences confirmed its suitability for oligonucleotide ID testing in the pharmaceutical QC environment. This procedure is robust and sensitive enough to meet internal requirements as well as those required of Vetter customers and the regulatory authorities.[5]

These and other ongoing studies have deepened Vetter's expertise in the field of oligonucleotides and special analytics.

How long does a measurement take? Is it difficult?

One analysis of one sample in a GMP environment may take less than an hour, depending on the parameters chosen. The temperature ramp rate is crucial for the time needed. Our preferred options include a temperature ramp rate of 10 °C/min, resulting in a 10-minute measurement.

The Cary 3500 UV-Vis system itself is easy to use. Any skilled laboratory technician or researcher can use the system and the procedure after short training.

Describe your experience working with Agilent.

When choosing an instrument for the Tm analysis in the ASL and QC, we selected Agilent and were among the first users of the Cary 3500 UV-Vis. We were also, to our knowledge, the very first users of the software for regulated environments following the compliance guidelines of FDA 21 CFR Part 11.

We have had a positive experience working with Agilent over the long term. We run various Agilent instruments in QC, including Agilent HPLC systems, as well as in the ASL, which uses the Agilent 7800 ICP-MS. Agilent has been a reliable and professional partner for service and questions; even with complicated issues, Agilent technicians were available within a short time and provided comprehensive support.

What do you think the future holds for this technology?

We will continue to study the performance of this ID test as new opportunities have emerged. We aim to establish and validate an analytical platform procedure based on the current knowledge which could enable a fast implementation of an ID measurement especially for new products or clients lacking a suitable analytical procedure for method transfer.

This goal includes a system upgrade of our instruments, which would further improve the precision of data analysis. We implemented and qualified the system right after market introduction in late 2019 and we still run the first version of the software that has the features needed for 21 CFR

Part 11 in regulated environments. The updated software provides now new built-in calculations for data analysis such as a smoothing function and enhancements to the derivative function. These can be saved within a method and applied automatically at the end of the data collection. We are currently working with Agilent to evaluate how these functions may be qualified on a system level rather than in product-specific procedure validations.

About the authors

Dr. Alexandra Heussner is an accomplished interdisciplinary scientist and laboratory manager within the Development Service of Vetter in Ravensburg, Germany. Dr. Heussner has contributed to our understanding of the toxicology of natural toxins and pharmaceutical drugs and supported method development and implementation by integrating biological sciences, information technology, and statistics. She received her PhD. in Applied Sciences from the University of Sunderland, UK, and after many years in academic research, she joined Vetter in 2016. She is currently involved in analytical procedure implementation, development, and validation with her team in the Analytical Science Laboratory. She is a member of the Parenteral Drug Association (PDA).

Dr. Melanie Zerulla-Wernitz is the Head of the Analytical Science Laboratory where she leads a diverse team of scientists combining natural and applied sciences. She holds a PhD. in natural science from the University of Konstanz, Germany. After many years in preclinical development Dr. Zerulla-Wernitz joined Vetter in 2004. Her career started in the quality control department, where she then progressed to Development Service. With a one-year excursion to Vetter Development Service in Chicago, she continues to lead the Analytical Science Laboratory and work on expanding its service and analytical portfolio.

References

1. Capaldi, D. *et al.* Strategies for Identity Testing of Therapeutic Oligonucleotide Drug Substances and Drug Products. *Nucleic Acid Therapeutics* **2020**, *30(5)*, 249–264. doi: 10.1089/nat.2020.0878.
2. Schuler, S. *et al.* Challenging Oligonucleotide ID Testing. *8th Annual Oligo Networking Event*, 23–25 Mar **2021**.
3. Schuler, S. *et al.* Emerging Therapeutic Oligonucleotides – ID Testing. *PDA UPS Conference*, 05–06 Oct **2021**.
4. Heussner A. H. Emerging Therapeutic Oligonucleotides – Analytical Challenges Accepted. *PDA UPS Conference* 05–06 Oct **2021**.
5. Heussner, A. H. *et al.* Investigating Tm Method Specificity Using Oligonucleotide Sequence Variants. *Journal of Pharmaceutical Science and Technology* **2021**, (submitted).

www.agilent.com/chem/cary3500uv-vis

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