Oligonucleotide Analysis Supports Exciting Therapeutic Approach

Waters ACQUITY UPLC-MS for oligonucleotide analysis

Technology: ACQUITY UPLC with ACQUITY QDa Mass Detector

A NEW CLASS OF THERAPEUTICS

The potential for oligonucleotides (oligos) to be used as therapeutics was first investigated around 30 years ago, and researchers achieved some early successes – the approval in 1998 of fomivirsen, for the treatment of cytomegalovirus retinitis in patients with HIV/AIDS, for example. At the end of 2017, more than 140 clinical trials of oligonucleotide drugs were in progress, but just six drugs had received regulatory approval. Some setbacks occurred in 2016 as a major pharmaceutical company withdrew one drug from trial and the FDA refused another approval. However, commentators still believe the DNA sense/antisense and siRNA sector has significant value and a very promising future. This is reflected in large growth seen in the oligonucleotide synthesis market, which supplies the raw material for oligo-based therapeutic and diagnostic development. One source estimates this market is growing at 12–15%/year and will be worth $1.92 billion USD by 2020, up from $1.08 billion USD in 2015.[1]

OPPORTUNITIES AND CHALLENGES

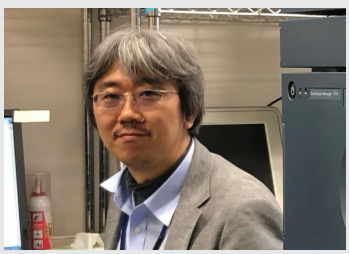
This continued optimism is based on several fundamental advantages of therapeutic oligonucleotides: for example, research has shown promising results across a wide range of medical conditions, and the potential to affect targets that cannot be effectively treated by small-molecule or protein therapeutics. In addition, when compared with small-molecule drugs and other large-molecule biopharmaceuticals, oligonucleotide products are theoretically much more straightforward to design, develop and scale-up to GMP manufacture.

The conventional laboratory of organic chemistry at Kobe University, Kobe, Japan.

WORKING WITH WATERS

Professor Kataoka has been working with Waters for the past 4 years, initially at Kochi University, and currently at Kobe University-Graduate School of Science, Technology and Innovation, as well as S-NAC, where he is the chief technology officer (CTO). The collaboration with Waters has grown as S-NAC's work on preparation of synthesized oligonucleotides has advanced and as they look to further optimize LC conditions.

Summarizing their working relationship, Professor Kataoka adds: *"The Waters team in Japan has always supported us very well, for example, when we needed advice on our choice of ion pair reagent, or information on how to export optimized report formats. It has been a very positive collaboration."*



Professor Kataoka, Research Associate Professor, Kobe University, Kobe, Japan.

Importantly, by interfering with RNA function at the cellular level, specific malfunctioning genes can be targeted, manipulated, silenced and/or modulated. Immune system modification is also possible, offering the possibility of treatment for a multitude of autoimmune disorders that are in many cases extremely challenging to treat with currently available drugs.

However, the regulatory environment is challenging. Regulators disagree about the classification of oligo therapeutics – with the FDA categorizing them alongside small molecules, while the EMA treats them as biologics. The result is two different approval processes. In response, manufacturers must currently consider the toughest regulations from each approach to put together a regulatory submission that will satisfy both.

SUPPORTING THE SECTOR WITH SYNTHESIS AND ANALYSIS

Professor Masanori Kataoka is Research Associate Professor at Kobe University-Graduate school of Science, Technology and Innovation, Kobe, Japan. The University has integrated natural science curricula, including advanced science and technology, with social science curricula, including entrepreneurship. This combination aims to cultivate future leaders who have the skills to promote R&D in advanced science and technology, as well as knowledge of intellectual property rights, industrial development, and market development.

Professor Kataoka is also Chief Technology Officer (CTO) at Shikoku Nucleic Acid Chemistry (S-NAC), a joint-venture company between: Kobe University and Shikoku TLO. S-NAC operates out of three facilities: a headquarters building at Takamatsu, with manufacturing in Kobe, and a sales office in Yokohama.

Professor Kataoka's department at Kobe University has more than 10 researchers working on the development of methods for oligonucleotide synthesis using nucleotide segment units that will be used for manufacturing nucleic acid drug substances. These methods offer high product-purity and competitive cost-performance.

With long experience of both research and commercial development of oligonucleotides, Professor Kataoka explains: *"Nucleic acid drug research is a very exciting field and I think it is important to have a vital, expanding pharmaceutical market for these new compounds. Our work at S-NAC in the synthesis and analysis of oligonucleotides – the building blocks for nucleic acid therapeutics – sees us supplying nucleotide trimer segments as substrates for further oligonucleotide synthesis, oligonucleotides as drug substance, and bioactive small nucleotides. We also work with pharmaceutical customers on process development for oligonucleotide manufacturing."*

As with any other organic compound, it is important to characterize oligonucleotides as they are synthesized. At S-NAC the analysis of impurities, and the measurement of protected nucleosides, mononucleotides, dimers, trimers, and oligonucleotides are routine.

The ultimate level of detail will, of course, be achieved by sequencing, but for everyday practice, it is sufficient to obtain the molecular mass of a molecule by recording its mass spectrum. Two methods have become established in this application: liquid chromatography (LC) coupled with either electrospray mass spectrometry (LC-MS) or matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (LC/MALDI-TOF).

To meet the analytical needs of their work, the well-equipped labs at S-NAC include a range of Waters instruments.

> "The ESI QTof systems in my university lab were overkill for the routine synthetic oligonucleotide analyses we wanted to do at S-NAC, so we decided to collaborate with Waters, and now at S-NAC we are using an ACQUITY UPLC/PDA/QDa workflow in Empower that we're very happy with."
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> PROFESSOR KATAOKA
> *Research Associate Professor, Kobe University*

Professor Kataoka continues: *"I decided to purchase the ACQUITY QDa when I was working at Kochi University in 2014, and when S-NAC was founded, we transferred the system to the new labs in Kobe. The ACQUITY QDa has an m/z scan range from 30–1250 and when combined with our UPLC system under Empower,™ it provides us with a simple, highly effective solution for analyzing the various components of our oligonucleotide synthesis process at S-NAC. We now test more than 200 samples per month, and analyze protected nucleosides, mononucleotides, dimers, trimers, and oligonucleotides."*

INTEGRATED UPLC-MS ON THE BENCHTOP

Waters® ACQUITY™ QDa™ Detector is a simple and cost-effective solution to add mass data into an existing UV-based workflow. With MS being such a critical analytical tool for the research, development and production of oligos, Professor Kataoka was quick to see the potential benefits of using the ACQUITY QDa:

> "Before the ACQUITY UPLC/PDA/QDa workflow in Empower was available, I would have used stand-alone MS instruments, but having an integrated system gives us a streamlined, rapid and very cost-effective approach. Importantly, the system is easy to operate, and its bench-top style does not require a large space but allows us to use it in the conventional organic chemistry laboratory."
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> PROFESSOR KATAOKA
> *Research Associate Professor, Kobe University*

Professor Kataoka continues: *"Empower is a user-friendly program, which allows even part-time technicians to master it in a short time. With Empower, they can complete an analysis in an hour. This is a huge time-saving, as previously, with the conventional LC-MS system, analysis would take one day."*

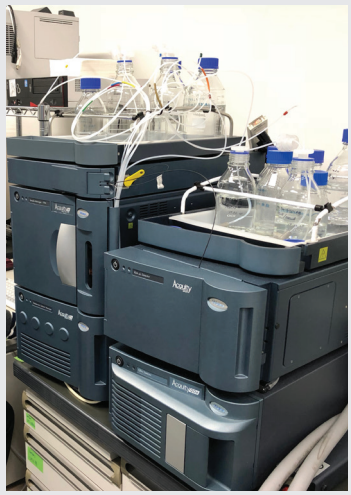
Professor Kataoka outlines how the system has exceeded his expectations: *"At first, I intended to use the ACQUITY UPLC/PDA/QDa workflow in Empower for a quick reaction check to confirm protection of nucleosides, but I found its performance was better than I had expected, so now we use the system for nucleotide trimer analysis and full length oligonucleotide analysis as well."*

Before the installation of the ACQUITY QDa Detector, we had significant difficulties detecting multi-valent ions of oligonucleotides. Professor Kataoka explained that those ions could not be confirmed with a good S/N ratio, and sometimes no peaks were detected in the measurement. In the case of monomer analysis, they found a limitation in protecting groups at nucleobases and ribose moieties. For protected trimer analysis, there was a limitation of molecular weight. In the case of oligonucleotides, there is a restriction to ionization, because multi-valent Ion peaks are sensitive to the ion pair reagent used. Professor Kataoka's group found that the best ion pair reagent for oligonucleotide analysis was HFIP-TEA. The ACQUITY QDa has allowed the group to overcome these limitations; an example of the detection of polyvalent ions of nucleic acid using the ACQUITY QDa Detector is shown in Figure 1.

Researchers at Kobe University-Graduate School of Science, Technology and Innovation, Kobe, Japan.

ACQUITY QDA DETECTOR

Since its introduction in October 2013, the ACQUITY QDa Mass Detector has allowed users to combine the resolution, sensitivity, and speed of UPLC technology with single quadrupole MS detection. Designed specifically for chromatographers with little to no mass spectrometry experience, it offers a simple yet powerful solution for rapid oligonucleotide ID confirmation and impurity analysis and process monitoring in routine laboratory environments – delivering robust and reliable performance, and walk-up operation for scientists any expertise level.



The ACQUITY QDA Mass Detector, Kobe University, Kobe, Japan.

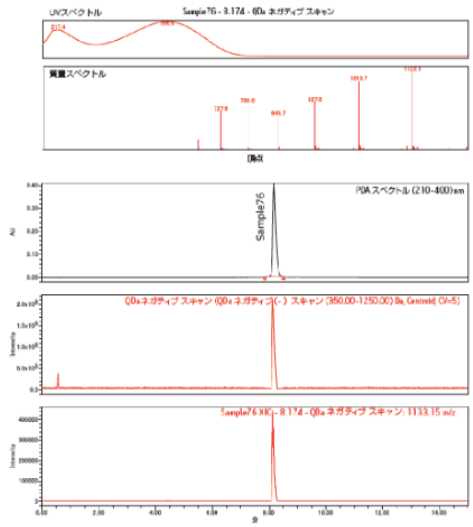


Figure 1. Detection of polyvalent ions of nucleic acid using Waters ACQUITY QDa Detector.

The ACQUITY QDa can be used to detect and measure nucleic acids up to 30 bases or more in length, which covers the majority of oligo based therapeutics now in development, as well as monomers. Nucleic acids with molecular weights of 10,000 daltons or more can be detected because with ESI they tend to adopt multiple charges, and the higher charge states clearly fall within the *m/z* scan range of the ACQUITY QDa, shown in Figure 2.

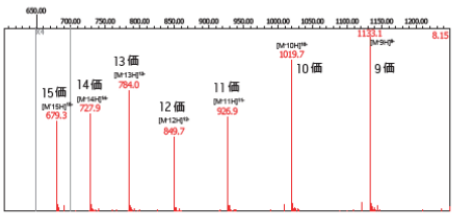


Figure 2. The ACQUITY QDa measures nucleic acids with molecular weight exceeding 10,000.

DEMONSTRATING THE VALUE OF THE ACQUITY QDA DETECTOR IN A PRODUCTION SETTING

Pharmaceutical companies partner with S-NAC for the development and supply of oligonucleotides. In this commercial environment, S-NAC is looking to be as efficient as possible as they deliver their high-quality products.

Professor Kataoka explains: *"We have improved production efficiency by adopting liquid-phase synthesis and developing nucleotide segments. The improved efficiency does not always reduce the analysis time but with the ACQUITY QDa we are able to achieve rapid qualification analysis of synthetic products, such as protected nucleosides, small nucleotides, trimer segment, and oligonucleotide. We are working towards our overall business aim – to increase efficiency in 2 digits."*

He continues *"GMP compliance is the next stage in our workflow."*

LARGE MOLECULES: THE CHALLENGE

Professor Kataoka sums up his experience with Waters and their equipment: *"MS analysis of large molecules can be difficult and getting MS peaks with good S/N values is hard. With the Waters ACQUITY UPLC/QDa in Empower, we can easily detect multi-valent MS peaks of large molecules, and through subsequent processing and charge state deconvolution, we can obtain the actual mass for each multi-valent species. This is achieved by exporting Empower MS data to another deconvolution program to calculate actual MS data. If the mass of UPLC-MS by MassLynx™ Software is measured, the deconvolution data is more easily obtained. Waters has several solutions for MS analysis of large molecules, so when it came to it, I trusted my MS analysis of oligonucleotides to Waters."*

Professor Kataoka plans to expand the use of the ACQUITY QDa, starting with quantitative applications to determine and monitor reaction conditions. *"I attended a recent Waters quantitative application seminar, and I am looking forward to starting this work in the future."*

"In summary, the ACQUITY QDa is suitable for analysis of oligonucleotides because it is easy to obtain multi-valent ions using ion pair reagents, and to confirm their identities via their set of m/z values. The ACQUITY QDa within Empower is easy to operate and its compact size allows us to use it in the small space we have available in the conventional laboratory of organic chemistry."

References

1. T Tredenick, 'Oligonucleotides: Opportunities, Pipeline, and Challenges', *Pharmaceutical Manufacturing*, 2016.

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