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BioTech Startup Advances Preclinical Studies for Topical Osteoarthritis Drug with Waters ACQUITY Premier System and Xevo TQ-XS

BriOri BioTech used cutting-edge technology from Waters to achieve the sensitivity and reduced carryover needed for analysis of a reformulated COX-2 inhibitor

Technology: Waters™ ACQUITY™ Premier System, Waters Xevo™ TQ-XS Mass Spectrometer, Waters ACQUITY Premier Columns

DRUG DEVELOPMENT AT BRIORI BIOTECH

In less than three years, BriOri BioTech has developed a next generation topical osteoarthritis drug with the potential to neutralize inflammatory pain and render opioid use obsolete for osteoarthritis of the knee, which afflicts over 18 million people in the U.S. alone. Osteoarthritis remains a highly prevalent disease in the elderly, and there are currently no approved treatments that can modify the disease course. For pain control, both oral and topical nonsteroidal anti-inflammatory drugs (NSAIDs), i.e., cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibitors, are frequently used to reduce pain and joint inflammation. However, oral therapeutic doses may cause gastrointestinal bleeds and cardiovascular events. Topical NSAIDs applied to the point of pain will reduce the systemic exposure to help reduce side effects while alleviating pain.

Backed by a team with more than 150 years of combined experience in successful U.S. Food and Drug Administration (FDA) submissions, as well as history in successful NSAID commercial development, BriOri BioTech's first project is working to repurpose a COX-2 inhibitor from an oral to a topical formulation. This product could potentially alleviate chronic joint pain, as well as reduce the need for opioids in certain patient populations. The global market for topical pain relief treatments was estimated to be $8.8 billion in 2019 and is projected to grow to $12.2 billion by 2027 at a CAGR of 5.2% from 2020 to 2027.[1]

Waters instruments have helped BriOri BioTech accelerate its preclinical studies for a topical osteoarthritis drug.

WORKING WITH WATERS

As a startup company, BriOri BioTech had a tight budget. As a result, its first laboratory was inside CEO Dr. Bruce Register's garage.

Having worked with Waters instrumentation throughout his career, Dr. Register initially bought a used Waters 600 HPLC, a system he was very familiar with.

As the company's needs grew and the laboratory expanded into a larger facility, BriOri BioTech continued to aquire more Waters instrumentation. Eventually, they became the first U.S. company to install the Waters ACQUITY Premier system coupled with the Waters Xevo TQ-XS Mass Spectrometer.

Previous experience in a molecular biology laboratory sparked the interest of Dr. Register in reformulating Vioxx (rofecoxib), a COX-2 specific NSAID that was voluntarily pulled from the U.S. market by Merck in 2004 once cardiovascular risks were noticed in a long-term clinical study. Since that time, however, new research has brought to light that all NSAIDs present similar cardiovascular risks. Dr. Register explains:

"We wanted to try and bring Vioxx back as a topical, because it's arguably the strongest analgesic ever approved by the FDA with the longest half-life. Rofecoxib was taken off the market in the United States because of the increased risk of cardiovascular events, but at the time we didn't know that every NSAID had the same [adverse effect] profile. Since then, the FDA has put a black box warning on all NSAIDs about the increased risk of heart attacks, strokes, and GI bleeds. It turns out that Vioxx was pulled prematurely, so that's why we feel confident in bringing it back as a dermal product to mitigate the risks."

However, reformulating rofecoxib as a topical product wasn't an easy process. Working with Waters technology, including the Waters ACQUITY Premier System and Waters Xevo TQ-XS Mass Spectrometer, BriOri BioTech has developed a formulation that it plans to move to clinical trials in 2022.

REFORMULATING ROFECOXIB

Rofecoxib was approved by the U.S. FDA in May 1999, and the drug was marketed by Merck & Co. under the brand name of Vioxx. Rofecoxib, a COX-2 selective NSAID that is GI sparing, is related to nonselective NSAIDs, such as ibuprofen and naproxen. Vioxx was available by prescription as both tablets and an oral suspension to treat osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, acute pain conditions, migraine, and dysmenorrhea.

Merck announced a voluntary withdrawal of Vioxx in 2004 after clinical trial results showed patients who took 25 mg of Vioxx every day for 18 months had an increased risk of cardiovascular problems, such as heart attacks and strokes, compared with those receiving a placebo.

Dr. Register started with a used Waters 600 HPLC System in his garage laboratory, which worked well for this early stage, despite the older analytical technology.

WORKING WITH WATERS

Support from Waters was vital during this period of rapid growth, and Dr. Register credits the camaraderie with his local Waters team as an element in the startup's early success.

It's a relationship that continues today, as the company looks towards the next steps in the drug development process.

Dr. Register explains:

"One of the things that I really like about Waters is that we've all become friends over the last two years. If they have some time, their specialists will come by just to see how we're doing."

" When Vioxx was withdrawn in 2004, there was a 30% increase in opiate prescriptions the following year. Nothing available, then or now, can substitute for the pain relief of rofecoxib. Tragically, up to 93,000 people died in the United States from drug overdoses in 2020 alone. That's unacceptable."

DR. BRUCE REGISTER
CEO, BriOri BioTech

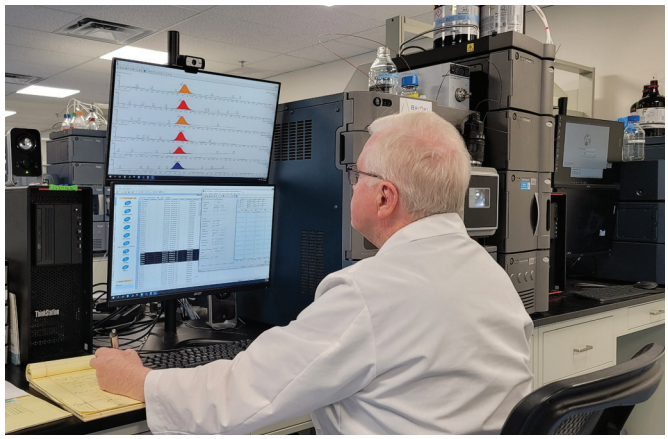
With firsthand experience with Vioxx and a background leading a laboratory at Merck, Dr. Register wanted to explore reformulating Vioxx as a topical ointment, thereby changing the way the drug is absorbed in the body and possibly reducing the risks. But working with rofecoxib was a challenge. He explains:

"The problem with rofecoxib is that it's totally insoluble. The only way that you can make it soluble is to put it in an organic solvent. And if there's any water around, it's going to precipitate and degrade. So, a topical product with rofecoxib is very, very difficult to make."

BriOri BioTech's first challenge was to develop a reformulation of rofecoxib as a topical ointment. Dr. Register started with a used Waters 600 HPLC System in his garage laboratory, which worked well for this early stage, despite the older analytical technology.

" I was at Merck for 20 years and spent 17 years in the lab, where we had a Waters 600 HPLC System. So, I knew the machine. It was great for doing diffusion studies through membranes, but not through skin. That's because the sensitivity on those old detectors is not at the level we needed for the later stages of this project. But if I'm putting 10 mg of rofecoxib in a sample ointment on a diffusion membrane, it's easy to quantitatively assay how much is getting through. The Waters 600 HPLC System worked really well for that."

DR. BRUCE REGISTER
CEO, BriOri BioTech



Dr. Register believes BriOri's key findings would not be possible without both the Waters ACQUITY Premier System and the Waters Xevo TQ-XS Mass Spectrometer.

Armed with those early results, BriOri BioTech used a contract research organization (CRO) to continue the diffusion experiments on human skin. Dr. Register explains how the CRO's results confirmed the potential of BriOri BioTech's initial formulation:

"The results blew us away. We were not expecting to get that lucky so soon. As a matter of fact, the ointment that we have settled on to move to clinical trials actually comes from that time in the garage testing it with the Waters 600 HPLC System."

WATERS SOFTWARE AND INSTRUMENTATION

As the laboratory expanded and moved to a larger facility, Dr. Register continued pre-clinical testing using a variety of analytical equipment. BriOri BioTech first leased the Waters ACQUITY QDa™ Mass Detector with Waters Empower™ Chromatography Data System (CDS) in the spring of 2020, and then later purchased a used Waters Xevo TQD Mass Spectrometer, as well as a new license of Waters MassLynx™ MS Software. Both the ACQUITY QDa Mass Detector and the Xevo TQD System were optimized for the detection of the proprietary small molecule analyte, the active component of the new rofecoxib dermal formulation under development at BriOri BioTech.

During the next phase of testing, Dr. Register coupled the Waters ACQUITY QDa Mass Detector with a Waters ACQUITY UPLC™ System, and the Waters Xevo TQD System with Waters ACQUITY UPLC H-Class PLUS System inlets. Those instruments significantly improved the limit of detection (LOD), which was determined to be 0.75 ng to 1 ng/mL. But the nature of rofecoxib continued to create some issues. During the tests, Dr. Register found a persistent level of carryover in the blank at 0.4–0.5 ng/mL. BriOri BioTech needed an LOD of less than 0.1 ng/mL for successful formulation development and animal studies.

" Now I had good sensitivity with the Waters ACQUITY QDa, and I'm looking at lower detection limits. I can see down to 1-2 ng/mL, but rofecoxib was sticking to the inside of the system. I could wash it 100 times, and it would not come off. The only way I could get it off is if I used isopropanol, methanol, hexane – a whole mixture of really nasty stuff. But you can't do that every other time you do an injection, and you certainly don't want to put it through your column."

DR. BRUCE REGISTER
CEO, BriOri BioTech

WATERS ACQUITY PREMIER SYSTEM

To overcome this, Waters personnel proposed the Waters ACQUITY Premier System and the Waters ACQUITY Premier Columns as a solution to BriOri BioTech's challenges, as both were launched around this time. The advanced system and columns are holistically designed to reduce analyte losses and improve repeatability for metal-sensitive analytes that interact with metal surfaces in conventional liquid chromatography (LC) technology.

Dr. Register has worked with Waters instruments for much of his career and now has the ACQUITY Premier and Xevo TQ-XS systems in his lab.

Non-specific adsorption of metal-sensitive compounds is an unpredictable challenge, leading to long system passivation times, chromatography with large relative standard deviations (RSDs), and broad peaks that can be a challenge to detect. The Waters ACQUITY Premier System features novel MaxPeak™ High Performance Surfaces (HPS) Technology, which effectively reduces non-specific adsorption losses due to metal interactions and provides reduced analysis times, improved peak shapes, decreased reagent costs, improved LODs and RSDs, reliable quantitation, and lower system downtime. Along with fast analysis times offered by UltraPerformance LC™ (UPLC) Technology, the Waters ACQUITY Premier System reduces the need for system passivation, which is often required to optimize assays for metal-sensitive compounds.

Dr. Register was intrigued, yet skeptical, that these new products could alleviate the observed binding and carryover of the small molecule analyte. However, the demo data showed convincing evidence that the system could detect the small molecule at 0.25 ng/mL concentration with no evidence of carryover in the blank. Dr. Register made the decision to purchase the Waters ACQUITY Premier System, and soon after leased the Waters Xevo TQ-XS Mass Spectrometer.

" I could get another order of magnitude better detection with the Waters ACQUITY Premier System. In fact, the first sample I ran I thought I had made a dilution error! Now, I get a LOD or 2–3 pg with an APCI probe. In addition, I can run a sample with 100 ng of rofecoxib and run a blank after that has background only. It works really, really well. It's a pretty remarkable system. I have nothing but good things to say about it."

DR. BRUCE REGISTER
CEO, BriOri BioTech

WATERS XEVO TQ-XS MASS SPECTROMETER

The addition of the Waters Xevo TQ-XS Mass Spectrometer allowed detection of the small molecule analyte and its metabolites at 1–2 pg/mL level, and again, showed no carryover signal in the blank. This capability allowed Dr. Register to make key decisions in the formulation development to mitigate a depot effect of accumulation of the small molecule in certain tissues, as well as its transfer into the systemic circulation.

"Our last step was now getting down to lower quantitation level for the mini-pig studies, as well as the upcoming human studies. And that's why I ended up with the Waters Xevo TQ-XS Mass Spectrometer. It's probably the most phenomenal machine that I've ever worked with in my life."

DR. BRUCE REGISTER
CEO, BriOri BioTech

The Waters Xevo TQ-XS Mass Spectrometer uses the StepWave XS™ ion guide that provides increased sensitivity for challenging compounds.

The new StepWave XS ion guide provides sensitivity that is reproducible for multiple injections and can provide reliable quantitation at the very lowest levels. It also ensures neutrals and gas load are passively removed for enhanced transmission, with the ions actively transferred into the mass analyzer, thereby improving sensitivity and robustness.

Using the Waters ACQUITY Premier System coupled with the Waters Xevo TQ-XS Mass Spectrometer, BriOri BioTech's research has indicated the company's product formulation could potentially outperform the competition.

These findings include:

- 3x better human skin penetration than competitors
- Area under the plasma drug concentration-time curve (AUC) and maximum concentration (Cmax) significantly greater than competitors
- Lower systemic exposure than oral dosing
- Accumulates in the synovium more than oral
- Superior pain efficacy to oral dosing at 8 hours and 24 hours
- Mini-pig Draize scores (skin irritation) all zero

Dr. Register believes these key findings in formulation development and animal study sample data interpretation would not be possible without both the Waters ACQUITY Premier System and the Waters Xevo TQ-XS Mass Spectrometer.

Dr. Register (left) credits the camaraderie with Waters Field Service Engineers like Jeremy Darcey (right) as an element in the startup's early success.

"First, I needed more sensitivity, and then I needed something that had less carryover, and then I needed something that was state-of-the-art to be able to analyze this data. Water's equipment did it all. It's a very difficult molecule to work with, particularly at low levels. With every step, as I needed better sensitivity or less carryover, Waters equipment got me there. And I was able to make it work to get the data that we needed to bring us to where we are today. The progression led me to buy what I have now, the Waters ACQUITY Premier System and the Xevo TQ-XS Mass Spectrometer."

DR. BRUCE REGISTER
CEO, BriOri BioTech

NEXT STEPS

With impressive results from the pre-clinical studies, BriOri BioTech is moving forward to obtain the financing to conduct clinical trials. Because rofecoxib was already approved by the FDA, Dr. Register is confident that a fast 505(b)(2) approval pathway is possible, which enables manufacturers to acquire FDA approval without repeating all of the pre-clinical and clinical safety studies of an already approved molecule. Additionally, the company recently received a notice of allowance from the USPTO for its first patent related to its novel formulation. That patent would block competitors from using any FDA-approvable solvents that are known to allow rofecoxib to be solubilized for a similar topical ointment until at least 2040.

Given the average time that is typical for drug development, the startup has moved impressively quick to get so far in such a short amount of time. Dr. Register gives credit to Waters instrumentation and personnel for their role in the process.

> " Our topical formulation is going to help millions of people who suffer from chronic pain. We've developed a product that appears to have greater efficacy than oral with a lower systemic exposure, which is remarkable. And I did this all in my garage with Waters equipment. Every time I look at the data for this product, it gets better. And we couldn't have gotten as far as we did without the Waters equipment. This instrumentation has been wonderful. We were able to get the data we needed reliability and quickly. I wouldn't have been able to do it without Waters."
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> DR. BRUCE REGISTER
> *CEO, BriOri BioTech*

References

1. https://www.alliedmarketresearch.com/topical-pain-relief-market

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