Cutting-Edge Analytical Technology Advances Arctic Marine Bioprospecting

Waters instrumentation and software solutions enable Marbio scientists to uncover marine microbes and new bioactive compounds with potential as novel drug leads, antibiotics, or ingredients for nutrition or cosmetic applications

Technology: Waters ACQUITY UPLC I-Class System, Vion IMS QTof Mass Spectrometer, UNIFI Scientific Information System

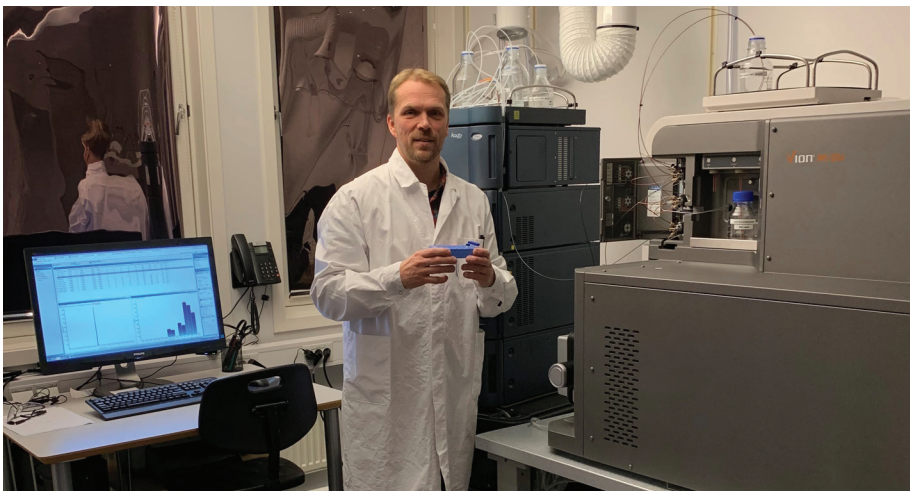
BIOACTIVE NATURAL PRODUCT SCREENING AT MARBIO

Since 2005, the Marbio analytical laboratory at the Arctic University of Norway in Tromsø has focused on high-throughput screening and natural products discovery by providing screening, isolation, and identification of bioactive natural products and molecules.

Located in northern Norway, the lab is ideally situated to prepare and screen fraction libraries from extracts provided by Marbank, a national marine biobank coordinating a network of marine collections. The mission of Marbank and the network is to provide national and international academia and industry with easy access to marine biodiversity, its associated data, and extractable products.

As part of this effort, Marbio scientists focus on marine bioprospecting – the systematic search for interesting and unique genes, molecules, and organisms from the marine environment with features that could be useful to society and/or have potential for commercial development.

Dr. Espen Hansen, Professor at the Norwegian College of Fishery Science, describes the founding principle of the Marbio laboratory: *"There was a political initiative from the Norwegian government focusing on marine resources in preparation for a shift in the economy away from oil. The government was trying to determine if we were able to exploit other marine resources as well. So, Marbio scientists began looking at bioactive products from the ocean with industrial potential."*



Dr. Espen Hansen in the Marbio Laboratory at the Arctic University of Norway.

WORKING WITH WATERS

Keeping abreast of analytical technology advances and new methodology in the field is vital to Marbio's cutting-edge research. As an academic institution, Marbio obtains new instrumentation and software through public funding sources and grants. As such, the laboratory focuses on acquiring technology that will advance its work. A Waters™ Vion™ IMS QTof was purchased in 2016 by a shared funding grant.

Dr. Hansen elaborates: *"It's not just buying an instrument to get a new instrument. We're looking for new technology that we can really benefit from. We're always keeping our eyes open to what's happening in the industry. We make an effort to learn as much as possible from colleagues and conferences, as well as instrumentation service and sales people."*

As part of this goal, researchers at Marbio are identifying natural marine products with the potential for drug discovery applications – such as antibacterial, anticancer, immunostimulants, anti-inflammatories, antioxidants, and diabetes treatment – as well as ingredients for nutrition or cosmetic applications.

Marbio's workflows rely heavily on the automation of sample handling, preparation, and analysis for the purification, bioactivity screening, and identification of natural products. The tight integration of robust screening assays, early identification of known compounds, and bioassay-guided purification of novel bioactive compounds is key to the lab's success.

Marbio scientists have worked closely with Waters to explore the potential of cutting-edge analytical technology to improve their success in arctic marine bioprospecting.

DISCOVERING UNKNOWN BIOACTIVE NATURAL PRODUCTS

The marine environment is a rich source of biodiversity, including microorganisms that have proven to be prolific producers of bioactive secondary metabolites. Arctic seas are less explored than warmer, more accessible areas, providing a promising starting point for Marbio to search for novel bioactive compounds.

> "Most of the research on marine biodiscovery focuses on tropical organisms. We identified this niche of studying bioactive natural products in cold-adapted organisms. That's our special focus. We started by working with marine invertebrates. Over the last three or four years, we have focused more on microorganisms as well, such as microalgae, bacteria, and fungi. They produce a lot of interesting compounds."
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> DR. ESPEN HANSEN
> *Professor, Norwegian College of Fishery Science*

Dr. Hansen collecting marine sediments for isolation of bacteria.

Marbio researchers identify and explore the potential of these bioactive natural products by isolating previously uncultured bacterial strains – some of which will produce new secondary metabolites. Dereplication is the first identification of the compound to see if they are known compounds or if there are potential new or novel metabolites.

Dr. Hansen describes this process: *"People often refer to it as finding a needle in a haystack. It's not a trivial task. The matrices that we are working with are very complex."*

The most common method of dereplication in natural product drug discovery employs mass spectrometry (MS) in combination with liquid chromatography (LC), as this combination is both sensitive and well suited for analyzing complex mixtures. Furthermore, when using high-resolution MS, the accurate mass of the compound can be used to calculate the elemental composition, which can then be used to search databases such as MarinLit, Dictionary of Natural Products, and SciFinder to identify known molecules.

Dr. Hansen explains: *"We do a prefractionation of the extracts, fractionating them into eight or so fractions, which we test for bioactivity. We use LC-MS to compare active and inactive fractions and pick out the bioactive ones."*

However, this approach will only recognize compounds that are identical to those in the databases, and any that are similar but non-identical to existing compounds will not be identified. Marbio's strategy to overcome this limitation is to use the Waters Vion IMS QTof to add information on MS/MS fragmentation in the dereplication process, as fragments will be characteristic for common structural features in a molecular class. These fragment data can be used to search MS fragment libraries such as Global Natural Products Social Molecular Networking (GNPS).

Dr. Hansen explains: *"When you are working with just the elemental composition, it is very hard to see the relationship between these molecules. But when you have the fragment data, you can see that you have specific fragmentation patterns that are similar even though the accurate mass of the target molecule is very different. So, we can see the relationship between molecules. That's very important to our work."*

ANALYTICAL TECHNOLOGY FOR MARINE BIOPROSPECTING

Discovering marine microbes and new bioactive compounds requires a variety of advanced analytical instrumentation and software technologies. Marbio previously invested in the Waters ACQUITY™ UPLC™ System and the LCT Premier™ mass spectrometer with MassLynx™ Software, and more recently added the Waters ACQUITY UPLC I-Class, Vion IMS QTof Mass Spectrometer and UNIFI™ Scientific Information System to the laboratory.

The acquisition of this new Waters technology improved Marbio's ability to use fragmentation patterns to identify new bioactive compounds. Waters HDMS^E data-independent acquisition mode on the Vion IMS QTof uses the selectivity of ion mobility to time-align precursor ions with their product ions, bringing the specificity of MS/MS to all compounds detected. This acquisition mode requires less set-up time, and because all the data is collected in one analysis, the need to reacquire is eliminated. Acquiring all data in one analysis combined with the added specificity from ion mobility provides Marbio researchers with the confidence to make better decisions, faster.

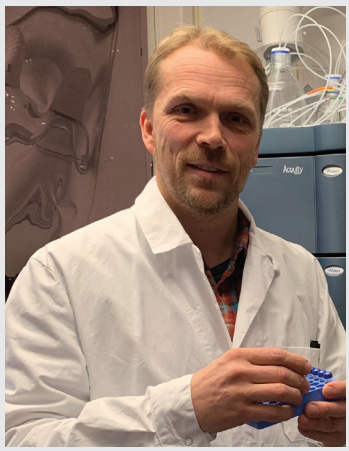
"Isolating the compound is the bottleneck in the lab because it's time consuming. Previously, when we selected our target compound, we were less certain that it was the right compound. So, we ended up with a number of cases where we isolated a compound that was not of interest because it turned out to be a trivial metabolite that didn't bring anything new. Previously, maybe 30-40 percent of the compounds were redundant and turned out to not be interesting at all. Since we've begun using the Vion, we haven't had any trivial molecules. It's had quite a dramatic effect. We have much more information and are more certain about the compounds we are working with. It's also very easy to run, even if you are not a highly skilled analytical chemist."

DR. ESPEN HANSEN
Professor, Norwegian College of Fishery Science

WORKING WITH WATERS

Dr. Espen Hansen, has been using Waters instrumentation for almost thirty years. He currently uses the instrumentation to assist him in the discovery of marine microbes and new bioactive compounds.

Dr. Hansen explains: *"I learned to do chromatography on a Waters instrument when I started studying in 1991. The Vion was purchased by a shared funding grant, and it is nice to allow the postdocs and PhD students to run their samples on it. It's an instrument with lots of fascinating physics, but you do not have to be too highly skilled to use it. This is a nice aspect. We also run samples from other universities in the consortium, as we are the only lab with the Vion."*



Dr. Espen Hansen.

The Waters Vion IMS QTof is driven by UNIFI Scientific Information System, enabling Marbio researchers to target, identify, and review results in a single software platform — and therefore make more informed decisions, faster. Waters UNIFI with Vion IMS QTof combines the separation power of LC, IMS, and high performance MS into a single solution that encompasses data acquisition, processing, visualization, reporting, and configurable compliance tools within a networked laboratory environment.

UNIFI offered Marbio the ability to significantly reduce the data processing bottleneck by providing rapid access to results through efficient data acquisition and processing steps. UNIFI's data mining and comparison capabilities also offered Marbio scientists the ability to understand results with an unparalleled capability to aggregate and manage data, as well as to build, create, and share comprehensive reports quickly and easily.

> "UNIFI is doing much more advanced processing of your data. You're looking at tables with the data, and the software is doing more data mining for you in order to pick up interesting compounds. We see the value of this heavy processing that is done in UNIFI."
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> DR. ESPEN HANSEN
> *Professor, Norwegian College of Fishery Science*

PUTTING THE TECHNOLOGY TO THE TEST

Marbio researchers have put their arsenal of Waters analytical instrumentation and software to good use in advancing the discovery of marine microbes and new bioactive compounds. For example, their recent work included the cultivation of a newly isolated arctic marine *Pseudomonas* sp. strain M10B774, which is affiliated with the *P. fluorescence* group, in four different media.[1]

Fractions of the culture extracts were screened for antibacterial activity against the pathogenic bacteria *Staphylococcus aureus, Enterococcus faecalis, Streptococcus agalactiae, Escherichia coli,* and *Pseudomonas aeruginosa* in a growth inhibition assay.

Dr. Hansen onboard the research vessel Helmer Hanssen.

Cytotoxic activity of the fractions was also evaluated against three cancer cell lines, human melanoma (A2058), human breast carcinoma (MCF7), and human colon carcinoma (HT29), as well as the non-malignant normal lung fibroblast cell line (MRC5).

Marbio researchers performed the study on a Waters ACQUITY UPLC I-Class and a Vion IMS QTof with an ACQUITY UPLC C_{18} Column. They used Waters UNIFI Scientific Information System software to process the data. *Pseudomonas* sp. was cultivated in four different media to activate biosynthetic pathways leading to the production of antibacterial and anticancer compounds. Culture extracts were pre-fractionated and screened for antibacterial and anticancer activities.

The active fractions were dereplicated using molecular networking based on MS/MS fragmentation data, indicating the presence of a cluster of related rhamnolipids. Six compounds were isolated using HPLC and mass-guided fractionation, and by interpreting data from NMR and high-resolution MS/MS analysis; the structures of the compounds were determined to be five mono-rhamnolipids and the lipid moiety of one of the rhamnolipids.

The project demonstrated the use of MS/MS-based molecular networking as a dereplication strategy to identify known compounds, their analogs, and related compounds. For the first time, five mono-rhamnolipids from a bacterium within the *P. fluorescence* group were characterized, including one new mono-rhamnolipid, as well as the lipid moiety from one of the rhamnolipids. Additionally, one fraction from three of the four growth conditions showed inhibitory activity towards bacteria and cancer cells.

Dr. Hansen comments: *"When you're starting an analysis like this one, you don't really know if you're looking for one of the smaller peaks or one of the really big peaks. So, you need that sensitivity. And it also means the dynamic range of the instrument is really important because the concentration varies so much between these molecules in the fractions. That's also where the advanced technology of the Vion is so valuable. You get the mass accuracy you need."*

NEXT STEPS

As Marbio researchers continue their bioprospecting research, the next step is commercializing the compounds they find to develop as novel drug leads, antibiotics or ingredients for nutrition or cosmetic applications. The Arctic University of Norway collaborates with local industry and European contacts, such as the Max Planck Institute for Medical Research in Germany.

Dr. Hansen elaborates: *"We have a relationship with the Lead Discovery Center (LDC) in Dortmund which is a center for translational medicine for the Max Planck Institute in Germany. We are a small university and do not go into drug development, but our partners pick up from here. It is difficult to count due to the number of multiple related compounds, but our Marbio facility is identifying between 30 – 50 compounds per year, that we are able to isolate and do follow-up bioactivity on. We are a productive lab, and the Vion has helped us to avoid wastage."*

In the meantime, Marbio scientists plan on continuing to push the boundaries of their Waters instrumentation and software to uncover marine microbes and new bioactive compounds.

> "We are academics working at the University, so we need to publish what we do. That's our focus here. But of course, we are trying to commercialize the compounds that we find, and we want to create new drugs as well as cosmetic and nutritional products and animal feed. The drug discovery process is a long and high-risk venture. We are trying to collaborate with local industry and we're working with biotechnology companies that can develop these compounds further."
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> DR. ESPEN HANSEN
> *Professor, Norwegian College of Fishery Science*

Dr. Hansen describes the future aspirations of the Marbio researchers: *"We're always looking at emerging technology to continuously enhance our work. We've had some discussions with Waters about some of their new technology. There are some very interesting things on the horizon that we're interested in trying. The natural products community is increasingly linking different types of data. We are doing more on metabolite profiles. We are also applying for funding to isolate compounds using supercritical fluid chromatography (SFC). It offers improved analytical scale, but also uses less organic solvents, so we can save the environment while doing our separations."*

References

1. Venke Kristoffersen, Teppo Rämä , Johan Isaksson, Jeanette Hammer Andersen, William H. Gerwick, and Espen Hansen. *"Characterization of Rhamnolipids Produced by an Arctic Marine Bacterium from the Pseudomonas fluorescence Group." Mar. Drugs* 2018, 16, 163.

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