BUILDING QUALITY ASSURANCE AND QUALITY CONTROL GUIDELINES FOR METABOLOMICS AND LIPIDOMICS

Perspectives on the community-wide effort

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TOWARDS QA/QC STANDARDIZATION IN MODERN ERA METABOLOMICS AND LIPIDOMICS

Contributions from Ian Wilson (Imperial College London) and Douglas Kell (University of Liverpool)

Metabolites and lipids play important biological roles, and studying them requires scientists to use advanced analytical and informatics platforms. As researchers around the world adopt metabolomics and lipidomics methods, challenges arising from a lack of standardized protocols for quality assurance and quality control (QA/QC) measures become more apparent. Leading researchers in this field are now developing guidelines for standardized QA/QC measures; these guidelines will address study design, instrument maintenance, and data acquisition. With these measures in place, researchers will obtain more reliable data that they can compare across workflows and laboratories.

While metabolomics and lipidomics have existed for decades, these fields garner more attention as researchers seek to comprehend organisms as a whole. By understanding metabolomes and lipidomes, scientists gain useful insights. "You get bigger changes in the metabolome than you get in the proteome or the transcriptome. A doubling of something in the transcriptome is a big thing; a doubling in the metabolome may just be within normal biological variation," said Ian Wilson, professor of drug metabolism and molecular toxicology at Imperial College London.

Compared to other -omics fields, accessing the metabolome and lipidome provides functional biological insights, with the most common analytical platform being liquid chromatography-mass spectrometry (LC-MS). However, interpreting the resulting data and ensuring that it is precise and reproducible is a challenge, often due to the sheer number of analytes measured in untargeted studies. "One of the biggest difficulties—this is true for all -omics methods—is the potentially very large dynamic range of all the metabolites," said Douglas Kell, research chair in systems biology at the University of Liverpool. "Few analytical instruments can cope with that kind of dynamic range."

In contrast, targeted studies typically investigate and absolutely measure the concentrations of small subsets of known compounds, simplifying their QA/QC. "If you move to targeted metabolomics, as opposed to untargeted, then the QCs change because you can use standard curves and internal standards," said Wilson. However, studies have shown that differences in procedures across labs affect even simple targeted assays.[1]

The large scale and long-term nature of many -omics studies further necessitates strict and uniform QA/QC measures to ensure reliability. "If you want to do hundreds or thousands of samples, you are going to have to use your mass spectrometers over many days, weeks, and months. You are going to have to calibrate them so that the control you did on one day maps itself back onto the control you did all those days back," said Kell. "The only solution is to run the same thing on different days, and adjust the appearance of the chromatogram and the mass spectrum."

Kell and Wilson co-authored a seminal paper outlining procedures for large-scale metabolic profiling, published in 2011.[2] In this document, they emphasized using identical pooled QC samples in untargeted studies where absolute analyte concentrations cannot be obtained during data analysis, an idea first brought forth five years prior.[3] "[Pooled QC samples] enable one to have some confidence in the fact that what you were measuring was actually a variation in the samples that you were putting in the machine, not just drift in the machine because you changed a column, or the source of a mass spectrometer, or any of the other hundred things that can change over time," said Kell.

Current QA/QC recommendations are either overly general,[4] geared towards targeted studies,[5] or seldom applied.[6] Experts are coming together to decide what best practices to recommend and how to disseminate this information and enhance compliance.[7-9] Scientific journals must ultimately play a role in this effort. "There needs to be an acceptance by journal editors that if the data is staggeringly important, then the science used to generate that data is thoroughly reviewed before publication," said Wilson.

Because of the influx of new researchers with their own protocols, instrumentation, and study species, it is clear that sharing standardized QA/QC guidelines across all metabolomics and lipidomics labs is critical for identifying data outliers and tracking instrument performance. This is a large undertaking that requires input from scientists in academia, industry, government agencies, and regulatory organizations.

THE BASICS OF QUALITY ASSURANCE AND QUALITY CONTROL

Contributions from Warwick (Rick) Dunn (University of Birmingham, UK) and Kim Ekroos (Lipidomics Consulting)

Metabolomics and lipidomics workflows are often complex and time-consuming, and variation can affect every step of the process, from study design to data analysis and interpretation.[1] Carefully implementing quality assurance and quality control (QA/QC) procedures saves valuable time and resources if researchers catch problems early and remedy them.

"With an appropriate QA/QC system, sometimes called a quality management system, you can have confidence that the data that you reported is of appropriate quality and meets minimum acceptance criteria. That gives confidence to paper reviewers, your peers, grant review panels, et cetera that [the data] are of high quality and that it's valid research," said Warwick (Rick) Dunn, professor of analytical and clinical metabolomics at the University of Birmingham, UK.

Scientists working in high-throughput analytical chemistry use QA measures to prepare for data acquisition even before they collect their samples. QA activities set the stage for the later analytical processes, ensuring that the processes will meet predetermined requirements for quality. Common QA processes include thoughtful experimental design, staff training, construction of standard operating procedures (SOPs) for biobanking, sample handling, and instrument operation, and instrument maintenance and calibration.[2]

Careful analytical platform monitoring is essential. "You have to assure that the instrument is performing," said Kim Ekroos, president of the International Lipidomics Society. "Even the same type of instrumentation from the same vendor can differ. You need the basic controls in place to know that this instrument is now performing as it should. You should also optimize the machinery or instrumentation based on the sample you are going to measure and the molecules that you are going to measure."

QC measures occur during and after data acquisition and address data quality requirements directly, giving scientists benchmarks to assess the reliability of their experimental data. These measures include using QC samples and standards that represent the biological samples in the assay to identify problems with the analytical process that could alter the data.[3]

Many QA/QC practices apply to both metabolomics and lipidomics workflows; however, differences occur in targeted versus untargeted studies. QA/QC is more straightforward in targeted studies because each analysis consists of a limited number of known compounds. Many researchers engaging in targeted studies follow FDA guidelines commonly used in regulatory pharmacology that were originally designed for targeted drug analysis.[4] However, these methods could be improved and tailored specifically for metabolomics and lipidomics. "Most of what is performed in the regulatory industry are targeted assays," said Dunn. "In regular metabolomics, it's more about untargeted metabolomics."

Regulatory QA/QC measures were not designed with -omics studies in mind and are not easily adapted for untargeted analyses that measure hundreds or thousands of unknown compounds. Some of the criteria used to determine quality in targeted assays are not appropriate for untargeted studies because researchers do not know the metabolites and lipids of interest until after they analyze the data. Scientists often use pooled QC samples, made by combining small aliquots of each biological sample in an analysis, to track variation in untargeted assays. "If the quality of the data for the pooled QC samples is poor, we assume that the quality of the data for the biological samples is poor, and we remove that metabolite from the dataset. We don't say the whole dataset is poor quality, we just remove those metabolites that are poor quality, and that helps improve both the data process and statistical analysis," said Dunn.

Researchers in metabolomics and lipidomics currently strive toward more defined QA/QC guidelines to meet the urgent need to ensure study quality. Defining QA/QC procedures for untargeted studies will allow for comparison of data across laboratories and encourage multi-laboratory studies. The communities are determining what the best practices should be going forward and how this information should be disseminated. "It is good to have a community-based type of discussion of what QA/QCs are and how these are to be developed further. We need to have training sessions, workshops at conferences, and so forth. This is something we need to really educate people on," said Ekroos.

DESIGNING EXPERIMENTS WITH QA/QC IN MIND

Contributions from Richard Beger (US Food and Drug Administration), David Broadhurst (Edith Cowan University), and Marina Wright-Muelas (University of Liverpool)

Metabolomics and lipidomics studies measure diverse metabolites while generating precise and reproducible data. Achieving both of these goals is a challenge, so implementing quality assurance and quality control (QA/QC) measures is imperative.

Good QA/QC starts at the beginning of a study. "You can bias a clinical study design by observing a small non-representative subset of people, such as a specific age or geographical location, and when you've completed the study any generalized interpretation to a greater population will be a biased extrapolation from which you may draw false conclusions," said David Broadhurst, professor of biostatistics and clinical data science at Edith Cowan University. "Conversely, you might only sample a small number, but look at a very big cross-section in terms of demographics, then that is potentially a very biologically diverse sample set, certainly for human studies. Therefore, you have less bias, but you're introducing statistical noise, or uncertainty, around any conclusions."

A solid experimental plan controls variability and includes standard operating procedures with well-defined sample collection, storage, and processing strategies. Additionally, staff training and instrument calibration are essential QA measures.

Because analytical instruments can introduce error into an assay, researchers use controls throughout a liquid chromatography-mass spectrometry (LC-MS) run. "Systematic error in metabolomics can be estimated through multiple measurements of QC samples. When the systematic error detected by the QC samples is greater than a certain amount, the cause of the error needs to be corrected before going forward with the analysis," wrote Richard Beger, branch chief of the Biomarkers and Alternative Models Branch, Division of Systems Biology, National Center for Toxicological Research, US Food and Drug Administration, in an email.

System suitability QC occurs prior to biological sample injection and determines whether the instrumentation performs as expected. "Often a blank gradient with no sample is run first to determine whether there are impurities in the solvents or contamination of the separations system including the column. Then a series of system suitability QC samples are used to determine correct retention time of standards within the acceptance criteria, chromatographic peak width and shape, mass accuracy of defined compounds within acceptance criteria, and sensitivity within acceptance criteria," wrote Beger. Next, scientists inject process or extraction blanks to identify impurities from the instruments, solvents and consumables, sample processing, and carryover of samples from previous runs. Before they inject biological samples, researchers often run several QC samples to "condition" their LC-MS platform; a process that primes the system and reduces error.[1]

Another source of error comes from instrument response drift as researchers inject samples into the LC-MS platform. "When we ran long experiments, we started to see instrument response drift—the attenuation of signal over time—as the column slowly dirtied up," recalled Broadhurst. "The signal was getting less as the sensitivity was getting less, but this change in sensitivity was clearly systematic and correctable using QC sample data."

Problems such as instrument response drift are apparent when comparing data from identical QC samples placed throughout a batch. For targeted assays, scientists compose QC samples from authentic chemical standards representing the target analytes and isotopically-labeled internal standards. For untargeted assays, experts suggest injecting identical pooled QC samples, made from biological sample aliquots, throughout the workflow.[2-4] "The aliquots should be taken from each of the collected samples during the initial sample collections and aliquoted into enough pooled sample aliquots to avoid additional freeze thaw cycles when they are used," wrote Beger.

To keep systematic error from biasing results, scientists should randomize the biological sample order. "Randomization is key, especially when you're running the samples through the mass spectrometer," said Marina Wright-Muelas, a research associate at the Institute of Systems, Molecular, and Integrative Biology at the University of Liverpool.

Finally, all studies should also include internal standards, long-term reference samples, and standardized reference materials. Researchers then correct measurement errors during computational analysis based off of the QC sample and standard results. "The simplest and quickest way of looking at the data is [with] PCA plots. [When] looking at the QC samples against your actual analytical samples, they should center around the center of the PCA plot," said Wright-Muelas. "If they're not clustering together, you've got a problem. You have to dig into the data and figure out who, what, when, and why."

The opinions expressed by Richard Beger in the responses to interview questions are those of the author and do not necessarily represent the views or policies of the US Food and Drug Administration.

USING STANDARDS TO MAINTAIN QUALITY

Contributions from John Bowden (University of Florida), Timothy Garrett (University of Florida), and Christina Jones (National Institute of Standards and Technology)

Researchers use chemical standards throughout their metabolomics and lipidomics workflows to assess their analytical platforms and data quality. In targeted studies, scientists also use standards for analyte quantification. Because of the large scope of untargeted studies, standards are particularly important for quality assurance and quality control (QA/QC) between experiments and laboratories.

"You are in this wild, wild West when it comes to the different workflows people use," said John Bowden, assistant professor of physiological sciences at the University of Florida. "Performing an untargeted analysis allows you to collect data across the entire mass spectrum. You're trying not to box things into a specific target. Then, during post analysis, we pick features out and make identifications. Having standards for that kind of analysis is important because it helps provide a level of confidence."

A number of companies produce labeled and unlabeled chemical standards for research and manufacturing. Scientists must choose the mix of standards that best suits their needs and apply it throughout their liquid chromatography-mass spectrometry (LC-MS) runs.

After testing blanks, experts suggest injecting a system suitability sample—a solution of authentic chemical standards for assessing system performance.[1,2] Ideally, the compounds cover a range of mass-to-charge ratios (m/z) and retention times for evaluating the expected analysis window. Researchers compare results to pre-defined acceptance criteria for those chemicals prior to injecting biological samples. "Samples are valuable, they cost time and money, and sometimes they're irreplaceable. Before you start any analysis on the system, you should run a system suitability test to make sure that everything is running properly," said Bowden.

In targeted studies, researchers typically add isotopically-labeled standards of their metabolites or lipids of interest to every biological sample. Because they know the standard concentrations, scientists can construct calibration curves to quantitate analytes. Internal standards cannot serve the same purpose in untargeted studies because the analytes of interest are unknown prior to data analysis; however, they are still essential. "You have to use some sort of standard that you add to every sample as a way to understand how the run is performing, and that tells you whether you can pass a run or fail a run. You need some assessment of variability to know that the instrument is performing right and that your extraction performed correctly. Then you can start making biological decisions," explained Timothy Garrett, associate professor of pathology at the University of Florida.

When every biological sample contains internal standards, researchers can monitor system performance in real-time and stop runs mid-batch if problems arise. Internal standards provide different information depending on when researchers spike the chemicals into their samples. "Extraction standards are added before you do anything to the sample as a way to measure how well you're extracting and how reproducible you are in the extraction process. Then the injection standards are added right before you're ready to inject," said Garrett. Internal standards added prior to injection help researchers adjust for instrument response drift that occurs over time after multiple LC-MS injections.

Long-term reference (LTR) samples and standardized reference materials (SRMs) help assess quality over time and across laboratories. Researchers use aliquots of the same LTR to normalize their data from batches on the same analytical platform. To compare data across laboratories, scientists purchase STRs developed by certified groups, such as the National Institute of Standards and Technology (NIST), that contain chemicals found in biological matrices. "You want a reference material that's the same matrix as the samples in your study," said Christina Jones, a research chemist at NIST. "There aren't always matrix-matched reference materials. If they don't exist, you have to go with the next best thing that's available to you."

As the metabolomics and lipidomics community grows, so does the need for diverse standard materials. Companies are currently developing additional reference materials that represent a variety of matrices. Yet, there is a long way to go before the community's needs are met. "A lot of people in this field create their own system suitability standards and their own long term reference materials…We are missing commercial ones that everyone can purchase," said Jones. "We are working to fill the gaps."

ESTABLISHING QA/QC REPORTING GUIDELINES

Contributions from Annie Evans (Metabolon) and Jennifer Kirwan (Berlin Institute of Health)

As metabolomics and lipidomics experts consider appropriate quality assurance and quality control (QA/QC) guidelines, they also discuss how researchers should report their QA/QC efforts to the wider community. Guidelines exist for QA/QC reporting in targeted studies; however, scientists inconsistently apply them, or they may be inappropriate for untargeted assays.[1,2] Several groups, including the Metabolomics QA and QC Consortium (mQACC), the Lipidomics Standards Initiative, and the Metabolomics Society, are currently deliberating which QA/QC measures should be shared and where to report them.[3] These reporting standards will likely be implemented across the entire metabolomics/lipidomics analytical process, including sample acquisition and processing, system calibration, QC standards and controls, and data analysis. Each QA/QC measure should be described with enough detail for researchers in other labs to reproduce the experiments.

"What you should report is still a very hot topic for discussion. As a bare minimum, we should be reporting markers that are an assessment of quality assurance of our experimentation," said Jennifer Kirwan, head of the Berlin Institute of Health Metabolomics Core Facility and one of the scientists working to establish reporting guidelines.

One arena scientists use for reporting their QA/QC efforts is data repositories, such as the Metabolomics Workbench through the National Institutes of Health and the MetaboLights database through the European Bioinformatics Institute. Scientific journals are another obvious choice. Every journal could provide the same reporting instructions agreed upon by various initiatives. Many researchers already include this information in their papers, but to varying degrees, and some journals require less rigorous reporting than others. Standardizing what is reported and where that information is found across all publications will enable readers to easily assess data quality and attempt to reproduce experiments with their own equipment.

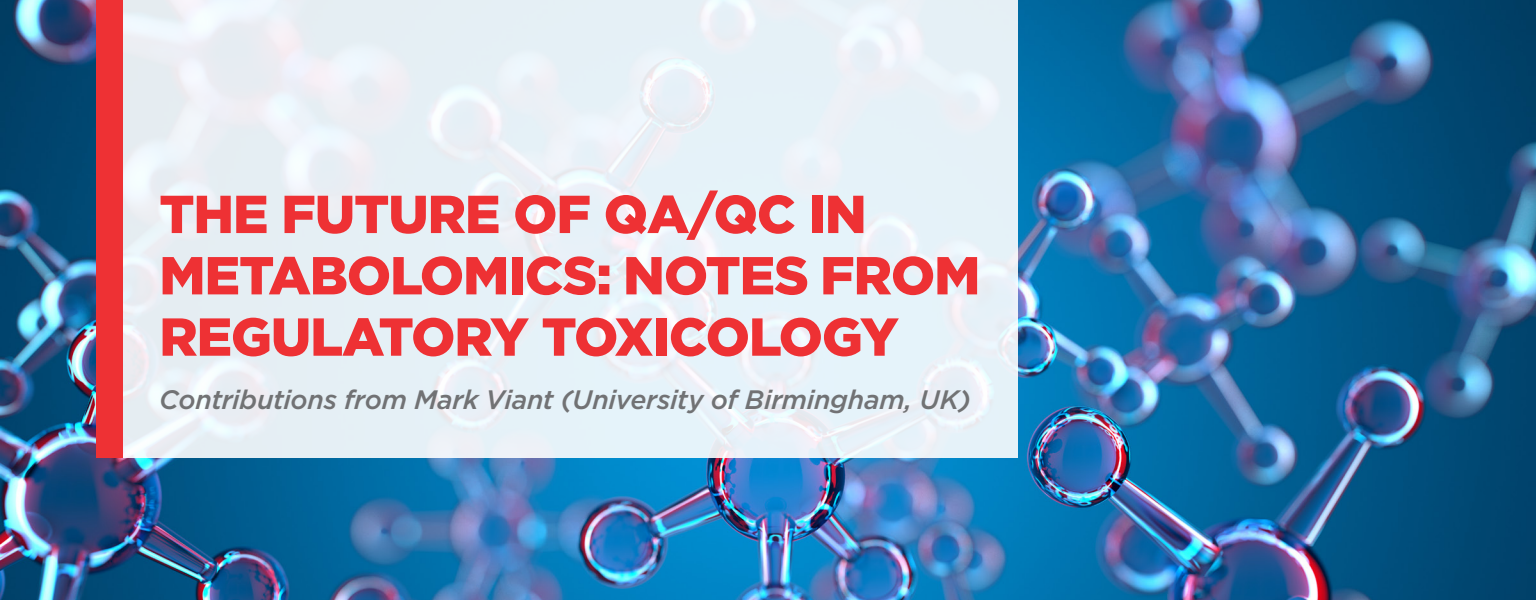
"Most reporting standards for quality control can go in supplemental [materials]. However, any interesting features of your quality control measurements that may directly affect the interpretation of the results should be reported in the main text," said Kirwan. "People should be open and honest about confounding factors and technical variation, which may be contributing to apparently clear-cut biological results"

Kirwan and Annie Evans, director of Research and Development at Metabolon, warned that recommending an overwhelming set of guidelines would decrease their adoption by some in the community. In fact, previously suggested guidelines have not been widely implemented.[4] "We don't really know why people haven't adopted them. Is it that the recommendations are too difficult? Is it because the publications were hard to get their hands on or there wasn't enough visibility?" wondered Evans. "Even if we set up some guidelines, we have to find a way to make it so that people can readily adopt them."

A strict set of guidelines may hinder scientists in smaller labs and those new to the metabolomics and lipidomics fields. "Labs have different facilities and different levels of funding, so the reporting guidelines should be flexible enough to allow people who are doing good work to publish that work," said Kirwan. With this in mind, the groups constructing the untargeted metabolomics and lipidomics reporting guidelines prioritize input from the wider community.

Evans, Kirwan, and other scientists are considering a set of minimal reporting standards, along with a list of best practice reporting standards. These options may enhance community acceptance and entice researchers to aim for best practice reporting whenever possible. "There are some people who are just getting into this science. If we set the bar so high, then you've essentially eliminated the ability of some of the people just getting into the field to meet the bar," said Evans.

Once the community decides on a set of reporting guidelines, the document will continue to evolve as technology changes. "We're thinking about this as a living document. We are going to set out the first set of guidelines after getting community input, and then in five years we need to look at them again," said Evans. "This is not something that's going to be put in stone. This needs to change and adapt with the science over time."



THE FUTURE OF QA/QC IN METABOLOMICS: NOTES FROM REGULATORY TOXICOLOGY

Contributions from Mark Viant (University of Birmingham, UK)

Experts in the metabolomics and lipidomics communities believe that clear quality assurance and quality control (QA/QC) guidelines are necessary. In 2007, the Metabolomics Standards Initiative published a series of papers outlining a set of standards and guidelines.[1] Now, many agree that researchers should update these standards, develop more rigorous guidelines, and take steps to improve their adoption within the community.[2,3] Groups involved in this process include the Metabolomics Society, the Metabolomics Quality Assurance and Quality Control Consortium (mQACC), the Lipidomics Standards Initiative, and the Metabolomics Standards Initiative in Toxicology (MERIT).

"One improvement that we need is an absolutely clear definition of what good practices in QA/QC are," said Mark Viant, co-chair of MERIT and professor of metabolomics at the University of Birmingham, UK. "New people moving into the field might say, 'Where are the clearly defined guidance documents and descriptions of how we should actually conduct rigorous QA/QC?'" A document that clearly describes best practices would guide both those new to metabolomics and lipidomics and established researchers.

Compliance with QA/QC best practices must increase across all laboratory types to enhance study quality; however, research groups have different incentives to comply with QA/QC guidelines. Those who focus on innovation move quickly from publication to publication and may have less time to replicate their efforts, while those in the regulatory arena already have structured protocols and reporting standards. "One of the challenges is, how do you actually bring the community forward as a whole? How do you encourage them? How do you incentivize them to use QA/QC and bring it into their experiments?" asked Viant.

Viant believes that regulatory scientists must participate in the effort to build metabolomics and lipidomics QA/QC guidelines, and a collaboration between mQACC and MERIT bridges the gap between regulatory science and biomedical research. The MERIT project brings together experts from industry, government agencies, regulatory organizations, and academia to develop guidelines for regulatory toxicology.[4] "We're trying to bring metabolomics and other molecular diagnostics into the chemical safety arena. People have been working in this space for 20 years, but we're trying to move it towards regulatory science, which actually means it would have to stand up in a court of law," said Viant.

The MERIT project works to develop metabolomics reporting guidelines for regulatory toxicology with the Organisation for Economic Cooperation and Development (OECD). Viant hopes that improvements in the regulatory field will trickle down to the wider community. "As the regulatory end gets better defined, hopefully that information can be fed back and made available to the community. Then the less-regulated community needs to be made aware of that and encouraged to take up and draw on some of that best practice."

Finally, QA/QC measures will improve with more metabolite and lipid standards. "We don't have the assays to identify extremely large numbers of metabolites or to quantify large numbers of metabolites. If we were looking into the future, we would certainly have improved assays for both of those," said Viant. "We don't even have metabolite standards for tens of thousands of metabolites and lipids. There will need to be a concerted effort by chemical supply companies to attempt to synthesize at least some of the critical missing metabolites." Prioritizing the production of analytes that are essential to certain fields, such as toxicology, biotechnology, or clinical medicine, could be the answer to addressing this large challenge.

> "One of the challenges is, how do you actually bring the community forward as a whole? How do you encourage them?"
>
> –Mark Viant, University of Birmingham, UK

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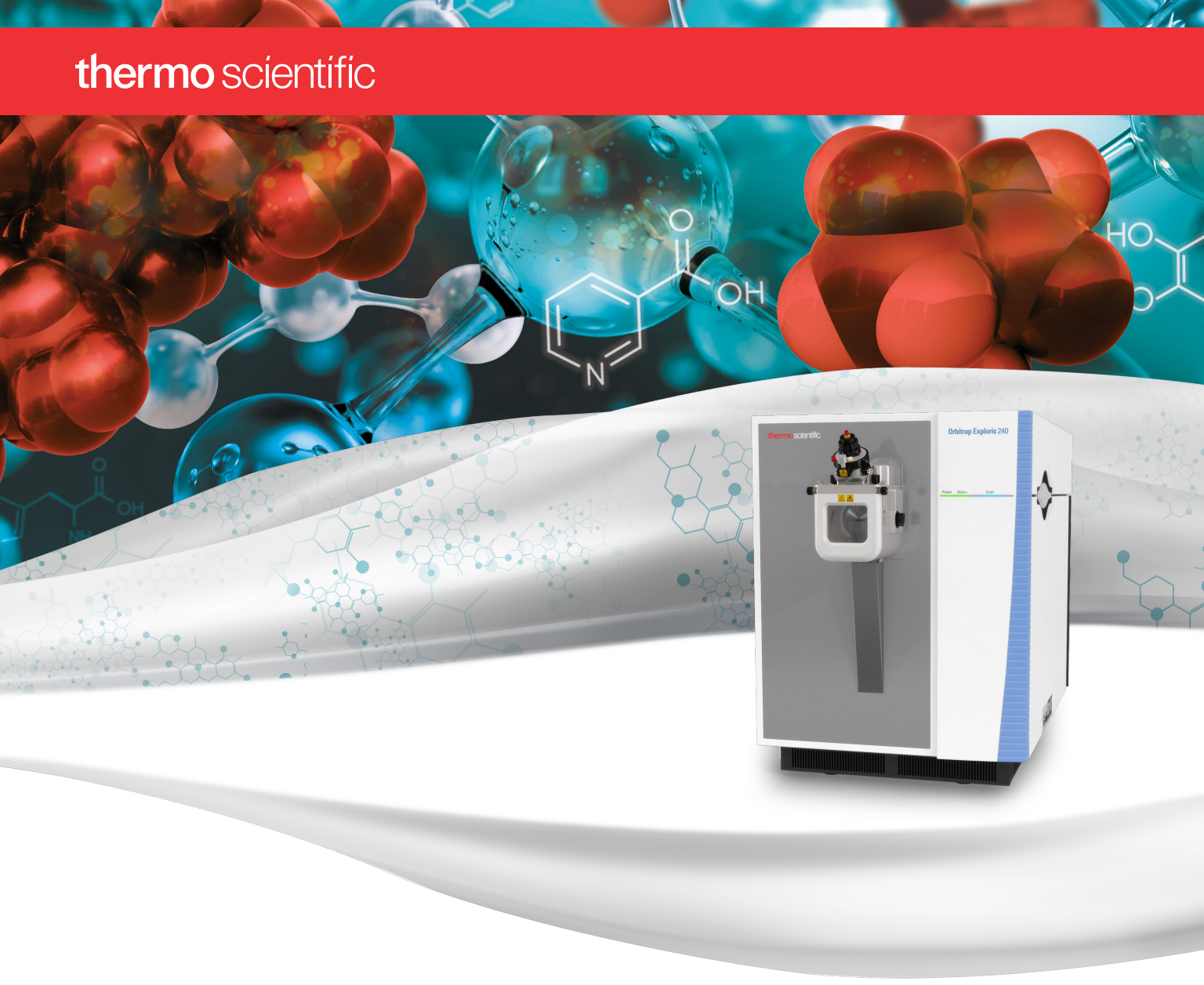

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