AGILENT CASE STUDY: EMPOWERING RESEARCHERS

# THE HUNT FOR HIDDEN LIPIDS
## AGILENT HELPS RESEARCHERS UNCOVER KEY BIOLOGICAL COMPONENT OF CELLS

**Zhi-Hong, Jiang, Ph.D.**
Associate Vice President, Professor, Macau University of Science and Technology
Director, Macau Institute for Applied Research in Medicine and Health

Sphingolipids can be found in the cells of all plants and animals. They can be found, but often aren't. It's almost as if these biologically important species are hiding, in very low concentrations, among other lipids (glycerides, for example) that appear in far greater abundance.

"With routine analytical instruments, we cannot detect these trace amounts of bioactive sphingolipids," says professor Jiang Zhi-Hong, who directs the Macau Institute for Applied Research in Medicine and Health.

Bioactive sphingolipids may be few and hard to find, but, Jiang notes, they are vital.

"They play an important role in the biological functions of the cells, so they are more important than other kinds of lipids," he says.

Some are closely related to drug resistance, others to diseases such as Alzheimer's and Parkinson's.

"The aim of our research is to discover more sphingolipids," Jiang says. "We want to more accurately, more comprehensively, analyze this component of our cells."

**The challenges have been plentiful, but Jiang and his team have overcome them with state-of-the-art instruments and powerful analytical software from Agilent.**

Biologically active sphingolipids exist in tiny amounts, but huge varieties.

"There are a lot of isomers homologues for sphingolipids," Jiang says. "If you cannot separate the isomers, you cannot quantify the sphingolipid. You cannot identify its structure."

So Jiang and his colleagues began looking for ways to improve accuracy and "unambiguously identify the structure of biologically important but low-abundance sphingolipids, which are highly diversified."

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*"Our approach facilitated unambiguous identification of several groups of potentially important but low-abundance SPLs that are usually masked by isotopic/isomeric species and hence largely overlooked."*

The solution: A combination of ultrahigh performance chromatographic separation with both quadrupole time-of-flight and triple quadruple mass spectrometry.

As Jiang and his collaborators wrote in the influential journal *Analytical Chemistry*:

"Our approach facilitated unambiguous identification of several groups of potentially important but low-abundance SPLs that are usually masked by isotopic/isomeric species and hence largely overlooked."

In fact, with the help of Agilent experts, the team was able to create a highly effective methodology that features improved sample preparation and optimized mass spec parameters.

**As a result, they were able to measure 86 individual sphingolipids in a single run—the highest number ever uncovered in PC12 cells.**

"The LC/MS instrumentation and software from Agilent were invaluable. Agilent's MassHunter software is very powerful and helped us to establish a database of sphingolipids. The qualitative analysis of this software also helped us identify the sphingolipids very quickly and very accurately," Jiang says.

"With the very powerful software and the good detection limit of the instruments, we achieved our goal of the accurate detection, identification, and quantification of trace amounts of sphingolipids."

Including sphingolipids that had never been detected before.

In its paper in *Analytical Chemistry 2014 Jun 17;86(12):5688-96*, the team noted that this improved method—which has been fully validated—will help researchers gather more reliable data, which could in turn lead to the discovery of important biomarkers.

"It has been proposed," they wrote, "that improved lipidomic measurements will become a standard clinical tool to ensure reliable diagnostics."

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