

# Case Study

## LC/MS<sup>n</sup> Ion Trap Technology for High-Throughput Drug Discovery

Accelerating pharmaceutical studies by using mass spectrometry to conduct comprehensive metabolism profiling assays

“The real impact of this program is not just screening more compounds faster and at a lower cost. Our assay gives greater insights into a compound’s liabilities and how to resolve those liabilities.”

Dr. Jonathan L. Josephs,  
Principal Scientist  
Discovery Biotransformation  
Research and Development  
Bristol-Myers Squibb Company  
Hopewell, New Jersey, USA



Like many people in drug discovery and development, Dr. Jonathan Josephs has spent the last decade wrestling with the huge potential of the new high-throughput, digitalized biology. The flood of information from various new “-omics” disciplines holds not only the promise of opening up biology, but also the pitfall of having information quantity overwhelm quality.

“Most people who approach discovery and development first ask, ‘what’s your throughput? How many people do you need to run that assay?’” said Dr. Josephs, principal scientist for Bristol-Myers Squibb (BMS). “People get overwhelmed with the data process and get focused on the information they can get most easily, not necessarily getting the information that is most valuable.”

For the past 10 years, BMS has repeatedly turned to Dr. Josephs for just that purpose, to find the most valuable information – identity, purity and metabolic profiles – of the most important compounds from the hundreds of thousands housed in BMS’ inventory. To meet that challenge, Dr. Josephs in conjunction with his colleagues, has developed four assays that have been critical in helping the drug company make the best choices possible when deciding which compounds should advance through the costly discovery and development pipeline.

As BMS has turned to Dr. Josephs, he has relied upon Thermo Scientific ion trap-based mass spectrometers to help researchers get more information about their compounds, save resources and accelerate research. Dr. Josephs has taken each new instrument’s advance in throughput



Thermo Scientific ion trap technology enabled BMS to accelerate the selection of candidates entering drug development.

and performance to create new protocols that more accurately characterize candidate compounds, including kinetics of metabolism and structural and quantitative assays.

### Structural Integrity Assay

Dr. Josephs’ joined the development group of BMS in 1996. In 2000, he transferred to discovery with a desire to improve the quality of compounds entering the development pipeline. One of the first assignments after joining the BMS drug discovery team was to find an easier way to guarantee the purity and integrity of compounds evaluated in profiling assays.

Confirming identity and purity was a challenge because the compounds being investigated encompassed a large volume of chemical space and yet would need to be investigated by generic methods. No one ionization method or polarity could cover all possible compounds. Therefore, a tiered approach was developed using pos/neg switching and both electrospray (ESI) and atmospheric pressure chemical ionization (APCI) modes. The process was automated by writing custom in-house software that utilized the OCX controls supplied with the Thermo Scientific Xcalibur data system.

Triple-quadrupole mass spectrometers needed to have their collision energy tuned for each analyte in order to obtain their fragmentation spectra, which aid in structural

assignment. Since most of these analytes are largely unknown, samples would need to be run multiple times over a range of collision energies, hoping to hit on the right energies for each analyte.

Since 1994, Dr. Josephs utilized the Thermo Scientific LCQ ion trap series of mass spectrometers and formulated the concept of “universal collision energy” which worked in tandem with the feature of Normalized Collision Energy, a patented technology only available on Thermo Scientific ion trap products.

By combining those features with Thermo Scientific Data Dependant acquisition, he found that universal fragmentation of all components of a sample could be obtained without human intervention in a single LC run.<sup>1</sup> Utilizing LCQ™ mass spectrometers, Dr. Josephs devised the new structural integrity assay in BMS, which enabled researchers to easily test purity, identify contaminants/degradants, as well as batch-process samples. The new assay ultimately helped increase confidence in the associated profiling data of compounds within drug discovery and accelerate the selection of candidates entering drug development.

This approach allows the automated acquisition of MS/MS spectra under universal conditions and enabled the development of a library of ~150,000 spectra that has utility in support of a number of other assays.

In 2003, the Thermo Scientific LTQ linear ion trap was introduced. A variety of new features on the LTQ™ model – including higher scan speed, better signal-to-noise ratio, improved sensitivity and spectral reproducibility, further enhanced the utility and application of these assays.

### Quantitative Assay: Metabolic Stability

Next, BMS asked Dr. Josephs to develop a quantification assay to analyze the stability of compounds in metabolic studies. BMS did not just want to confirm the structure of the compounds in their inventory; they also wanted to understand how these compounds were metabolized.

At the time, ion trap instruments were not typically used for quantitative analyses. Dr. Josephs discovered a way to combine the faster full-scan MS<sup>n</sup> data from the LTQ linear ion trap mass spectrometer and new data analysis software he developed with his colleagues to create a quantitative metabolic stability assay.

“Many people believe you can’t quantify on ion traps, but in our experience we’ve proven that in drug discovery using Thermo Scientific instruments you can quantify with the sensitivity, precision and accuracy that is required in the assays that we conduct,” Dr. Josephs said.

This assay gave researchers a quantitative view of their compound using mass spectrometry for the first time, without adding cost or time to the assays, and with less equipment and fewer people. Despite

advances in triple-quadrupole instrumentation and software, the BMS Discovery Group has found that Dr. Josephs’ method is still faster.

“We realized that the linear ion traps with Ion Max source would be an ideal instrument for doing both the structural integrity assay and the quantitative assay, because we could get our MS/MS spectrum in a data-dependent fashion at no extra cost or time, and we could obtain those at a single collision-energy because of the universal collision energy,” Dr. Josephs said.

### Quantitative Assay: Metabolic Profiling

Once Dr. Josephs figured out a way to quantify a compound’s metabolic stability, the natural next step was to devise a way to quantify its metabolites. An automated, accurate metabolic profiling assay would allow the investigation of more compounds, faster and with greater depth than before.

In drug discovery, a compound’s metabolic profile consists of its metabolites and their quantities over a kinetic time course. Having this profile affords a greater ability to make accurate decisions about a compound’s liabilities and provides insights into how to fix them. However, getting an accurate profile is analytically very difficult. The standard approach involves a low-concentration assay giving the rate of disappearance of the parent compound, and a second incubation, at much higher concentration, to identify which metabolites are formed. To get accurate enzyme kinetic information, the two measurements should be made in the same incubation tube.

Dr. Josephs wanted to devise a way to do these measurements in one assay; the LTQ linear ion trap, with its higher sensitivity and faster duty cycle, gave him the ability to do it. Dr. Josephs’ team first developed a high-concentration assay (30 μM), and performed a 30-minute LC run to identify the major metabolites, and quantify them by UV. To determine the metabolic profile, Josephs’ team performed a second low-concentration assay (0.5 μM) in parallel, and tested the sample at different time points using a two-minute LC gradient method using SRM detection. Creation of a “metabolite standard

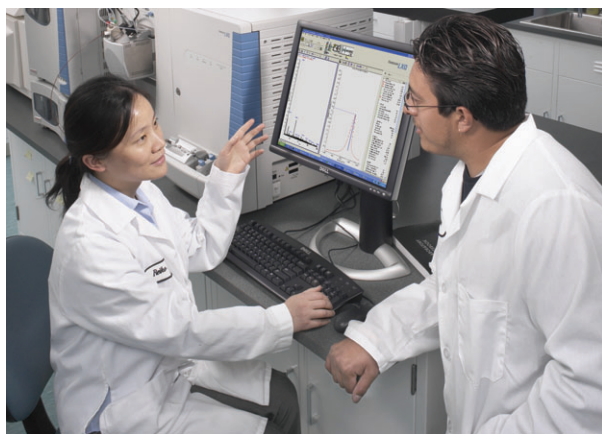
solution” by dilution of the 30 μM incubation sample allowed correlation from the high-concentration UV chromatogram to the mass spectrometer response and, therefore, quantification of the identified metabolites. Quantifying the parent compound and the metabolites in the same incubation tube was ideal for enzyme kinetic studies.

To date, Dr. Josephs’ metabolic profiling assay is the only assay in use that links the kinetics of a parent compound disappearance and the formation of metabolites in the same incubation.

“The real impact of this program is not just screening more compounds faster and at a lower cost. Our assay gives greater insights into a compound’s liabilities and how to resolve those liabilities,” Dr. Josephs said. “We don’t have to look at as many compounds because we can look more deeply at the compounds we have for desirable properties.”

Dr. Josephs has further enhanced this assay by integrating the newest Thermo Scientific LTQ Orbitrap instrument into his workflow. High resolution, accurate mass data, particularly in the MS/MS mode, greatly facilitates structural elucidation of the detected metabolites. Initial studies on the benchtop Orbitrap™ instrument, the Thermo Scientific Exactive, in conjunction with U-HPLC suggests that U-HPLC/high resolution/extracted ion chromatograms may be a simpler alternative to SRM quantitation of metabolites over multiple time points.

1. *PSB 121, Enhanced Fragmentation of Small Molecules in Thermo Scientific LTQ Series Linear Ion Trap Mass Spectrometers Using Stepped Normalized Collision Energy*, Thermo Fisher Scientific, 2006.
2. *An integrated LC-MS strategy for preclinical candidate optimization*. Jonathan L. Josephs and Mark Sanders, in *Integrated Strategies for Drug Discovery Using Mass Spectrometry*, Edited by Mike S. Lee, 2005 John Wiley & Sons, Ltd.



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