

## A New Era in the Structure Elucidation of Natural Products Using the CapNMR Probe

Mark O'Neil-Johnson<sup>1</sup>, Eliane Garo<sup>1</sup>, Jin-Feng Hu<sup>1</sup> and Jean-Luc Wolfender<sup>2</sup>

<sup>1</sup>Sequoia Sciences, Inc. 11199 Sorrento Valley Rd, San Diego, CA and <sup>2</sup>University of Geneva, Laboratory of Pharmacognosy and Phytochemistry, Geneva, Switzerland

Natural product chemistry has traditionally been a long and time-consuming process for any drug discovery research program. From extraction, isolation and purification of a biomass to the eventual structure elucidation process of the active compound, creating real value for today's high throughput screening (HTS) program in a large pharmaceutical company is a challenge. Sequoia Sciences' has proven that its rugged HPLC extraction and isolation methodologies for purified natural product fractions fit right into today's HTS platforms. Sequoia has also overcome the barrier of structure elucidation on minute quantities, i.e. microgram quantities of active natural product compounds, by efficiently manually coupling the CapNMR probe with off-line HPLC separations, creating a successful natural product discovery platform that approaches scales necessary for traditional screening quantities.

In recent years, the development and commercialization of new probe technologies has greatly increased NMR sensitivity. These include Nano probes spinning at the magic angle, HPLC-NMR probes with flow injection or flow probes with reduced coil volumes, 5 mm Cold Probes and CryoProbes, 3 mm MicroCryoProbes and HPLC-NMR with either CryoFlowProbe or an interchangeable flow cell (IFC) Cold Probe. Each of these new probe introductions have continued to push the sensitivity barriers in the acquisition of NMR data. However, limitations still exist. Limited data set acquisitions due to reduced sensitivity or poor quality of heteronuclear data, high costs of deuterated solvents, poor separation of compounds on HPLC columns due to sample overload or the prohibitive costs of CryoProbes for a majority of labs, has limited full use of these probes in their respective configurations. Using the flexibility of the CapNMR probe coupled with off-line HPLC separations, we have created a robust system that minimizes data acquisition limitations for the rapid isolation and structure elucidation of active compounds of limited mass.

We are currently using the CapNMR probe for all of our NMR data acquisitions. It has enabled us to acquire 1D proton and 2D Gradient COSY spectra on quantities of less than 5 micrograms with very few non-deuterated artifacts in approximately 5 minutes and 1.5 hours, respectively. HSQC experiments are routinely acquired on sample of 30 micrograms in approximately 5 hours. What is well understood in natural products drug discovery programs is that these NMR data sets, along with a low-resolution mass spectrum, typically lead to a chemical class and potentially a proposed structure. A proposed structure, as well as available information on its chemical class, biological activity, synthetic accessibility and therapeutic index will typically enable a company to properly allocate resources to pursue this as a lead. Acquiring NMR data on sample scales equivalent to biological screening amounts has vastly accelerated the process and has reinvigorated the use of natural products as a rich source of chemical diversity.

To illustrate the integration of the outputs from our purification process with the capabilities of the CapNMR probe, we describe the sample preparation of an extract of *Greenwayodendron suaveolens* and the isolation of a novel antibacterial compound, suaveolindole. Detailing the high-throughput generation of a natural products library containing this compound and the high-throughput isolation and miniaturization the structure elucidation process utilizing the efficiency of ACD Labs' Computer Assisted Structure Verification software as well as integration of other analytical techniques (FT-MS) will be presented. In addition, a comparison of the CapNMR probe data with data previously acquired via traditional HPLC-NMR on an isolated set of compounds from the *Gentianaceae* species will be illustrated.