

# Automating metabolic inhibition assays - on a nanoscale

AstraZeneca is one of the world's leading pharmaceutical companies focused on producing effective medicines that make a real difference in important areas of healthcare. In today's environment, the constant streamlining of laboratory processes in order to increase efficiency and save valuable resources is an important aspect for such companies, and taking advantage of the latest advances in automation technologies can help to get ahead.

Brett Litten is a senior DMPK scientist and automation specialist at AstraZeneca's Alderley Park site in Cheshire, UK, where he is jointly responsible for new technology and the design and delivery of various automated *in vitro* assays and *in vivo* related processes within the department. The company has significantly invested in Tecan's technologies over the years and one of Brett's latest projects has been to integrate a Nanodrop™ II eight-channel nanopipettor from Innovadyne Technologies with a Tecan Genesis RWS™ 200 Workstation and Tecan Safire™ microplate reader.

This configuration is being used to increase throughput in the cytochrome P450 inhibition assay. In this assay, the activity of five major human cytochrome P450 enzymes (CYPs) is determined using a number of substrates, which are metabolized to fluorescent products. Inhibition of the CYPs results in a decrease in the amount of fluorescence metabolite formed and, by using a range of test drug entities, the potency of inhibition (IC<sub>50</sub>) can be assessed. Brett integrated the Nanodrop™ with the Tecan workstation in order to miniaturize this assay for use with 1536-well plates, as he explained: "We required a higher plate density to help reduce assay costs and increase throughput. The Nanodrop™ allows us to use a 1536-well plate such that a greater number of drug entities at different concentrations can be tested against the major P450 enzymes per assay. Furthermore, using 1536-well plates leads to a reduction in the number of times the assay is run per week for our current research purposes and, in turn, frees up more resources. The process is fully automated, including fluorescence analysis with the Safire<sup>2</sup> plate reader, so our researchers can get on with other tasks during the assay."

Innovadyne's Nanodrop™ II is a small scale, high accuracy, high precision nano-dispenser, which is able to efficiently handle very small volumes of liquid, as low as 100 nL. The instrument will also happily dispense microliter-range volumes, so it has quite wide-ranging uses.

"The Nanodrop™'s attraction is that it extends the current usable range of our Tecan workstations, which are generally speaking of a larger volume criterion. Due to the Nanodrop™'s small size, it is easy to install into an existing robot platform," said Brett. "It is very simple to integrate, and uses a combination of traditionally robust syringe aspiration coupled to Innovadyne's micro-solenoid dispense valve assembly for delivering accurate quantities of liquid."

In order to run the assay, the 1536-well plates are loaded on to the Tecan workstation with the manually prepared reagents and enzyme solutions. The workstation's Gemini™ software is then used to execute the assay, controlling the various sequences for the Nanodrop™'s processes, including spiking the wells with 100 nL of test compound. Once the Nanodrop™ has prepared the plates, the Tecan robotic manipulator (RoMa) arm transfers them to the Tecan incubator, also housed on the system,



The Nanodrop™ II



Brett Litten and the Genesis 200 workstation



The Nanodrop™ II and the Safire<sup>2</sup> on board the Genesis 200

for necessary preincubations and incubations. The RoMa arm can then remove the plates after the required reaction time and place them on the specified site on the Nanodrop™ for the next steps before finally transferring the plate to the Safire<sup>2</sup> for detection.

The Safire<sup>2</sup> is a double monochromator plate reader that is capable of reading 96- to 384- and 1536-well plates in fluorescence, fluorescence polarization, absorbance and luminescence modes. The metabolic inhibition assay uses fluorescence substrates that are metabolized by the enzymes and produce a fluorescent signal, which can be quantified to indicate the potency of the test compound to inhibit the enzyme-substrate metabolic reaction.

“We chose the Safire<sup>2</sup> because we needed a detection system that could read 1536-well plates in a very short space of time, so we could minimize evaporation and keep the automated process as fast as possible,” Brett explained. “We have actually integrated it so it is physically connected to our Tecan workstation and is directly accessed by the RoMa arm. The Safire<sup>2</sup> is the fastest unit I’ve ever used for reading plates, capable of reading 1536-well plates in just 37 seconds! We read our 1536-well plates using several fixed wavelength methods to accommodate the different excitation-emission wavelengths required for our assay.”

Once the plate is read, the raw fluorescence data are stored to a text file

and automatically transferred to a network server from where analysts can process the data to generate inhibition IC<sub>50</sub> values.

Since Brett’s original bespoke integration of the instruments, Tecan and Innovadyne have been working together to produce off-the-shelf systems where the Nanodrop™ is fully integrated with Tecan’s Freedom EVO® liquid handling workstations. These systems will be suitable for diverse assay processes requiring smaller volumes – for further information please contact Matthew Webb, Tecan UK Limited.

*Nanodrop™ is a trademark of Innovadyne Technologies, Inc.*

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