Automated pH measurements and solubility screening of candidate compounds in pharmaceutical development

Scientists at AstraZeneca UK in partnership with Tecan are developing a fully automated solubility screening platform, a Freedom EVO® 200 liquid handling workstation integrated with a tailor-made data capture system, a novel pH flow cell carrier and a small footprint HPLC system.

AstraZeneca's Early Development Group, part of Pharmaceutical Research and Development at the Alderley Park site in Cheshire, UK, receives many compounds made or isolated by AstraZeneca's Discovery Group and assesses them for their suitability for development. This requires large-scale solubility screening at variable pHs and, until recently, this was performed using time-consuming and costly manual processes. AstraZeneca recognized that, to improve efficiency, automated solubility screening was needed and ideally should require no operator intervention once the screen has begun. Suitable fully automated systems were not available off-the-shelf at that time and it was then that the scientists approached Tecan.

Rod Kittlety, Senior Research Scientist in the Early Development Group, explained, "We had a number of criteria for our automated solubility screening system. It needed to measure solubility at different times and over a large dynamic range (1 µg/ml to more than 20 mg/ml) and the pH to an accuracy of 0.1. A system using centrifugation rather than filtration for solid separation was essential, because solubility measurements at concentrations below around 50 µg/ml can be affected by compound-specific absorption to filters. Another requirement was the incorporation of an HPLC system which could handle a number of different methods thus allowing the stability of the compound in question to be assessed during the experiment. We wanted to eliminate operator intervention after the process started, and to be able to control the temperature over a range from 4 °C, which is the minimum storage temperature, to 37 °C, the physiological temperature. Tecan worked with us to develop a system that satisfies all these requirements."

AstraZeneca's solubility screening platform consists of a Freedom EVO 200 workstation equipped with a specially-designed automated pH measuring device and integrated with liquid handling (LiHa) and robotic manipulator (RoMa) arms, an automated centrifuge, and a small-footprint HPLC system. The flow-through pH cell is controlled by software that integrates with Freedom EVOware®, allowing calibration, measurement and pH stability analyses. An integral data capture system converts the PC into a configurable data acquisition device for automated calibration and measurement of pH, then returns the captured data to Freedom EVOware for export. The benefits of this adaptation include stable pH readings and correction of sample pH readings with calibration data, in 96- and 384-well formats. For most compounds, the amount of substance needed for analysis is small; two 50 µl aliquots of diluted sample injected sequentially give good data for most buffers.

"The equilibrium solubility of a compound increases as it starts to ionize, and this is dependent on pH, so

Rod Kittlety loads the carrier which holds the pH calibration buffers

measuring the pH of the suspension accurately is essential when assessing the pH-solubility relationship," said Rod. "We needed this adaptation to obtain all these pH measurements at defined time intervals. We use four buffers with defined pHs from 1.68 to 10.0 to calibrate the electrode which means we can cover the physiological pH range within which pharmaceutical formulations are required to work. It is important to measure substance solubility over a sufficiently wide range of pHs so that solubility under physiological and formulation conditions can be assessed."

The pH calibration is done before each solubility time point. The solubility screening platform allows sample pH measurements to be carried out while sample aliquots are centrifuging. The centrifuged samples are then diluted and aliquots of these are injected into the HPLC system; the solubility of 36 samples at three time points is measured usually up to 48 hours, and many pH measurements are automatically performed during the process.

"Solubility results from test compounds from the automated system agree very well with those from manual screens," Rod continued. "The automated system gives us the precision we need over the required pH and solubility ranges, and the instrument is very robust. I have done about 3,000 sample injections on the pH system while setting it up and it works really well. The system has a great deal of potential for allowing us to do many of the things we normally do in the laboratory, but automatically and in a smarter way. For example, we are now using the system for a plasma precipitation screening assay to measure the level of active compound in plasma samples, and we can complete this in just half an hour using the Freedom EVO platform, compared with almost a whole day when performed manually."

Research application, not for clinical diagnostic use.

For more information, visit
www.tecan.com/analytics