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THE ROLE OF LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY IN MEDICINAL CHEMISTRY

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INTRODUCTION

Confirming the identity and quality of new chemical entities is a major challenge facing the pharmaceutical industry. Maximum efficiency is essential for laboratories challenged by throughput requirements and the management of data from a variety of systems and users.

Liquid chromatography with mass spectrometry has become the standard technique for confirming the identity and purity of drug discovery compounds to support high throughput screening (HTS), optimization, and physicochemical property profiling of these compounds. Medicinal chemistry is an iterative process and requires rapid turnaround times. High throughput solutions together with advanced data handling software must be employed.

In this application note, we look at various solutions, including sub-2 µm column particle sizes, fast scanning mass spectrometers, and new software to assist the medicinal chemist in five key areas:

- Screening
- Confirmation
- Purification
- Compound profiling
- Optimization

METHODS AND DISCUSSION

Screening

It is important to verify the identity and purity of a compound before early activity studies. Chemists need to be sure they have synthesized the expected compound. Large numbers of compounds may be created, so it is necessary for this screening to be high throughput. Because only a small amount of material is synthesized, the screening must also consume as little material as possible, while generating a diverse amount of information.

Figure 1. The ACQUITY SQD with the Sample Organizer plus PDA and ELS detectors.

Samples were analysed on a Waters® ACQUITY UPLC® System with a Sample Organizer. The column was an ACQUITY UPLC BEH C_{18} (1.7 µm, 2.1 x 50 mm) run at 30 °C. The injection volume was 5 µL. Compounds were separated using a generic water/acetonitrile gradient that was 1.1 min long.

Detection was done with an ACQUITY UPLC Photodiode Array (PDA), ACQUITY UPLC Evaporative Light Scattering (ELS), and SQ Mass Detector with an ESCi® source for ESI/APCI switching. Plates were logged into and processed with the OpenLynx™ Open Access Application Manager for MassLynx™ Software.

By using an ACQUITY UPLC System with the Sample Organizer, we were able to analyze 3840 samples in under 7 working days on a single column. On a traditional HPLC system, this would take approximately 27 working days, assuming a 10-minute run time.

The ESCi source on the mass spectrometer allowed the chemist to gather data in both electrospray and APCI (with positive/negative switching) modes during the same injection. In this way, the maximum amount of data was generated with a minimal amount of sample.

The open access interface allowed the user to log in the sample plates while providing a minimal amount of information. A series of methods, each including gradient conditions, MS conditions, and processing parameters, was designed by the system administrator. The user simply chose a method from this list, imported their sample lists, and placed their microtitre plates in the indicated positions.

The samples were then analyzed and the data was processed. Once processing was finished, the data was automatically copied to a file storage PC. From here the users could do further processing, if desired. A report file was also generated from the processed file and converted to pdf. This facilitated storage of the results in a database.

Confirmation

Exact mass experiments permit elemental composition determinations of unknowns or confirmation of a suspected elemental composition. This allows the medicinal chemist to confirm identities of known compounds, to rapidly identify unknowns, and to characterize complex sample components.

Samples were analyzed on an ACQUITY UPLC System. The column was an ACQUITY UPLC BEH C_{18} (1.7 µm, 2.1 x 50 mm) run at 30 °C. The injection volume was 5 µL. Compounds were separated using a generic water/acetonitrile gradient that was 1.1 min long.

Detection was done with an ACQUITY UPLC PDA and an LCT Premier™ XE Mass Spectrometer with an ESCi source for ESI/APCI switching. Samples were logged into the system using OpenLynx Open Access and processed with MassLynx OpenLynx with i-FIT™ exact mass processing.

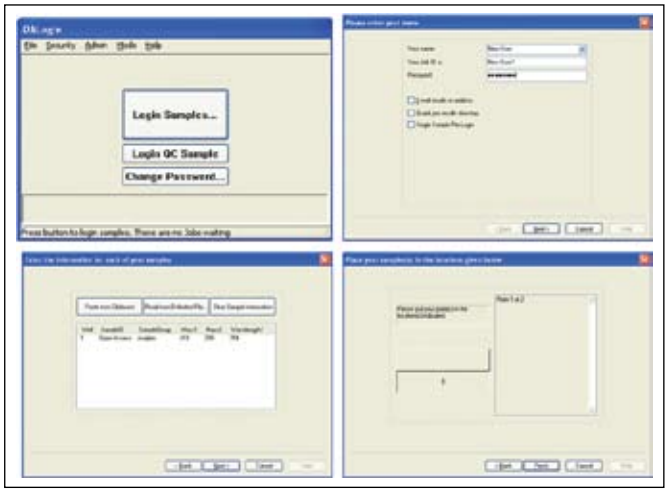

Figure 2. OpenLynx OALogin plate login wizard.

A fast generic liquid chromatographic method was designed to provide excellent selectivity without compromising either chromatographic resolution or speed of analysis. To obtain such an analytical method, UPLC® in conjunction with oa-TOF MS detection was employed. With this analytical system, identification of the anticipated samples, isomers, and possible impurities with mass accuracy deviations less than 5 ppm from the actual were obtained using LockSpray™. With such high accuracy data, the calculation of elemental compositions for each of the analytes was possible.

Subsequent elemental composition results were produced using the i-FIT algorithm, which takes into account the distribution of the spectral isotopes for the compounds of interest and employs novel data interpretation to simplify results lists returned.

The Open Access interface allowed the medicinal chemist to log in the samples while providing a minimal amount of information. The results, including a pdf report showing the most probably elemental compositions, were then made available to the chemist.

Purification

Having a pure building block is important for controlling the synthetic reactions and successfully making a pure target. A pure target is critical for understanding the results of screening and building quality structure/activity relationship (SAR) information.

Reverse-phase HPLC has been successfully applied to the different aspects of the medicinal chemist's process. It is capable of purifying milligrams to multiple grams in a single system, and can be configured to automatically process hundreds of samples. The results can provide high purity and recovery of the desired compounds with minimal user intervention.

Samples were analyzed on a Waters AutoPurification™ System, including a 2545 Binary Gradient Module, 2767 Injector, and Collector, and a System Fluidics Organizer (SFO). The compounds were purified on an XBridge™ Prep C_{18} ODB™ column (5 µm, 19 x 50 mm) run at room temperature.

Detection was done with 2996 PDA, ELS, and 3100 mass detectors. Fraction collection and processing was done with the FractionLynx™ Application Manager. Compounds were separated using 5-minute gradients that were chosen by the AutoPurify™ functionality of FractionLynx.

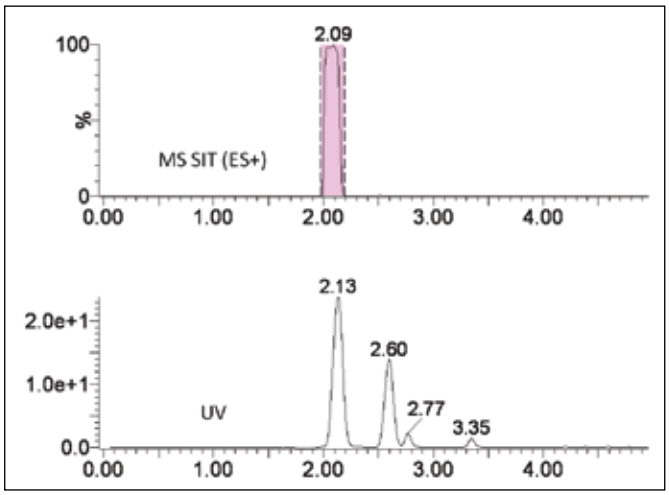

Figure 3. MS and UV chromatograms showing targeted mass and impurities.

A rapid LC/MS method was developed for the analysis of a medicinal chemistry library. The MS data confirmed the presence of the target compound and its retention time from a high resolution LC separation with a 1-minute cycle time. The retention time corresponded to a percent organic solvent at which the compound eluted.

Based on this correspondence, a focused purification method for a 19 mm I.D. column with 5 micron particles was selected to maintain the analytical resolution. The isolated target was then separated by LC. The original analytical methodology was then used to determine the new purity for each compound collected.

By logging in their samples just once, the medicinal chemists were able to get a purified product along with reports showing the initial and final purities.

Compound profiling

In an effort to avoid clinical failures, there is an emphasis across the pharmaceutical industry on examining pharmacokinetic and safety profiles earlier in the drug discovery process. Assays are developed in order to select compounds with the highest probability of becoming successful drugs based on preferred pharmacological properties. This step includes extensive testing for the absorption, distribution, metabolism, excretion, and toxicity (ADMET) and physicochemical properties of a compound.

Samples were analyzed on an ACQUITY UPLC System with a Sample Organizer. The column was an ACQUITY UPLC BEH C_{18} (1.7 µm, 2.1 x 50 mm) run at 30 °C. The injection volume was 5 µL. Compounds were separated using a generic water/acetonitrile gradient that was 1.1 min long.

Detection was done with an ACQUITY UPLC PDA, a ACQUITY UPLC ELS and a Quattro Premier™ XE Mass Spectrometer with an ESCi source for ESI/APCI switching. MS conditions were optimized using the QuanOptimize™ Application Manager. The samples were processed using the ProfileLynx™ Application Manager. Properties analyzed included solubility, logP, microsomal stability, and CHI.

Figure 4. ProfileLynx browser showing results of solubility experiment.

Early screening of physicochemical properties (PP) is an integral process for modern drug discovery. Typical PP profiling practices include properties such as solubility, stability (pH and metabolic), permeability, integrity, etc. The critical factor to consider in PP profiling is throughput. The bottlenecks to throughput include MS method optimization for a large variety of compounds and data management for the large volume of data generated.

An automated UPLC/MS/MS protocol was developed that not only allowed for automated MS method development and data acquisition, but also allowed data generated from multiple tests to be processed by a single processing method, all in an automated fashion. As a result, the physicochemical profiling process was significantly simplified and throughput increased.

The column manager bypass channel allowed users to easily switch to direct flow injection analysis for compound optimization without sacrificing one of the column positions. Chemists can choose the optimal conditions and chemistry for their compounds as the column manager is a thermostat-controlled oven with temperature regulation from 10 to 90 °C and has automated switching for four columns.

Optimization

Once a hit is generated through library screening, optimization of the compound of interest takes place. This step involves multiple repetitions of chemical modification of the hit to develop compounds with desired properties. Chemists need to know as soon as possible that these reactions are proceeding as desired.

Samples were analyzed on an ACQUITY UPLC System with a Sample Organizer. The column was an ACQUITY UPLC BEH C_{18} (1.7 µm, 2.1 x 50 mm) run at 30 °C.

The injection volume was 5 µL. Compounds were separated using a generic water/acetonitrile gradient that was 1.1 min long.

Detection was done with an ACQUITY UPLC PDA, ACQUITY UPLC ELS and an SQ Mass Detector with an ESCi source for ESI/APCI switching. Single samples were logged into the system using OpenLynx Open Access and processed with the OpenLynx Application Manager.

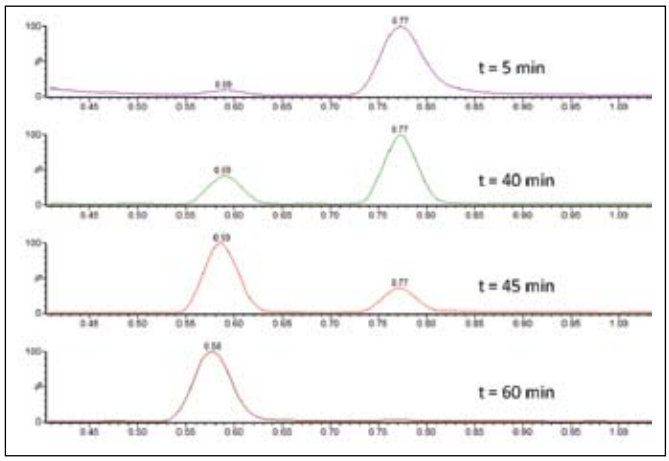

Figure 5. Chromatograms from various times during a 60-minute reaction.

During the compound optimization stage of a discovery cycle, medicinal chemists are not only interested in determining the key structural features responsible for activity and selectivity, but also what structural changes need be made to improve these characteristics. Because the reactions necessary to bring about these changes may take a long time, chemists need to be sure they are progressing as expected.

By using a walk-up UPLC/MS system, chemists were able to quickly and easily monitor their reactions, noting the relative amounts of starting materials and products. They were also able to note the formation of any side products and make the necessary alterations to minimize these in their reaction protocol.

CONCLUSION

We were able to increase throughput and data quality by combining UPLC with a variety of detection techniques and software solutions.

- **Screening:** By combining the speed of the ACQUITY UPLC System with the capacity of the Sample Organizer, we were able to nearly quadruple the screening throughput of the lab, without sacrificing data quality.
- **Confirmation:** With the Open Access interface, medicinal chemists were able to confirm the elemental composition of their compounds, with minimal instrument training. The i-FIT algorithm simplified the final exact mass determination by reducing the number of possible elemental formulas.
- **Purification:** We were able to use analytical LC/MS data to tailor the purification method to maintain the analytical resolution.
- **Compounds profiling:** The determination of physciochemical properties was simplified with the use of the ProfileLynx Application Manager, which automated the calculations of solubility, logP, metabolic stability, and CHI. The combination of the Column Manager and QuanOptimize facilitated the development of optimal MS/MS method.
- **Optimization:** Chemists were able to quickly and easily log in their samples to determine the progress of the reaction. They were able to see the results of the analyses within minutes.

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