INVESTIGATING THE IMPLEMENTATION OF CONVERGENCE CHROMATOGRAPHY FOR MEDICINAL CHEMISTRY AND PROCESS DEVELOPMENT LABORATORIES

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Michael D. Jones[1], Sean M. McCarthy[1], James McKearin[2], Jon Kremsky[2], Andy Aubin[1], Paula Hong[1], and Margaret Maziarz[1]
1. Waters Corporation, 34 Maple Street, Milford, MA 01757; 2. Prime Organics, 25 Olympia Ave, Woburn, MA

INTRODUCTION

Synthetic chemists work in a wide range of industries generating compounds for both final products as well as key intermediates. A thorough understanding of the optimal synthetic route allows chemists to make knowledgeable decisions related to increasing purity and yield. In pharmaceuticals, medicinal chemistry departments also require high throughput and robust instrumentation. However, the workflow is highly dependent on the instrumentation, which ideally can allow a pharmaceutical organization to compartmentalize new entities into compound libraries. This can further increase the chances of bringing a target through to the lead optimization process.

In this presentation, we will describe the use of Ultra Performance Convergence Chromatography for monitoring several synthetic processes as performed in a medicinal or synthetic chemistry laboratory. The benefits of this new technology in expanding the current analytical capabilities of the synthetic chemist will be illustrated. Specific examples will illustrate the benefits of implementing CC in simplifying workflows, analyzing structurally similar compounds and determining requirements for an orthogonal approach, all in order to aid better decisions to drive compounds to market.

METHODS

Instruments

UPC²

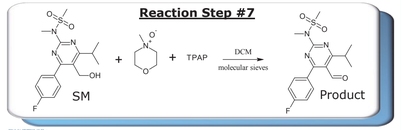
Instrument:	ACQUITY UPC² with 6 column capacity
Detectors:	PDA and SQD2
Mobile Phase A:	CO_2 (tank, medical grade)
Modifier B:	15 mM ammonium formate/1% formic acid in MeOH
Column:	*See figure captions*
Injection Vol.:	0.5 µL
Column Temp.:	Achiral columns: 50 °C Chiral columns: 35 °C
Flow Rate:	2.0 ml/min
Gradient:	*See Figure Captions*
ABPR pressure:	1885 psi
Wavelength:	254 nm
CDS:	MassLynx with OpenLynx and OA Login
Make up Flow:	0.1 % NH_4OH in MeOH

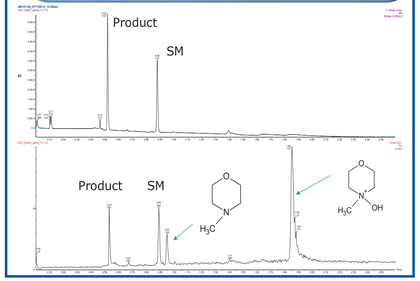
SQD2

Capillary (kV)	2.00 kV
Cone (V)	20.00 V
Extractor (V)	3.00 V
Source Temp.	150 °C
Desolvation Temp.	500 °C
Cone Gas Flow	50 L/Hr

Importance of Mass Spectrometry

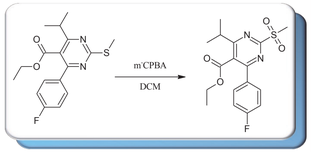
In some cases, the starting material, the degradants of a starting material or other byproduct of the reaction may not contain a chromophore. These non-chromophore containing constituents, when not detected, can lead to a lack of understanding of the synthetic process. This lack of understanding can hinder the troubleshooting of poor yield and impure final products.
In the example below, the seventh reaction step in the rosuvastatin synthesis was monitored. The MS data clearly detects reduction impurities of one of the starting materials. The presence of these impurities may inhibit yield of further reactions downstream.

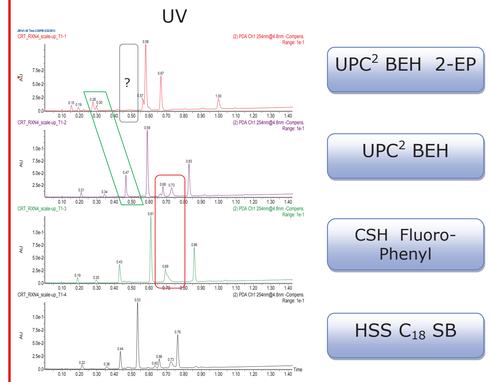




Enhancing 'Intra-Technique' Selectivity

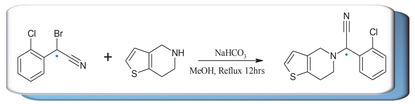
The fourth reaction step in the synthetic route for rosuvastatin (*right*) was monitored by UPC²/MS. There is a selectivity difference observed for this mixture when screening columns as evidence by UV and MS detection (*below*). With MS, we tracked the elution order of m/z=304 Da as seen with the blue arrow. We also identified two isobaric species (m/z = 362 Da) which were resolved on the 2-EP and BEH columns (*which were not detected by UV)* as depicted by the blue rectangle. By utilizing UV/MS, we can see another isobaric species m/z = 265 Da which splits into 2 peaks when using a UPC² BEH 2-EP column. The separation of these isobaric species are not observed on the other columns as depicted by the green parallelogram.



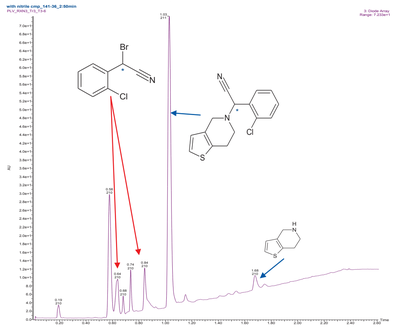


Importance of Screening Chiral Columns

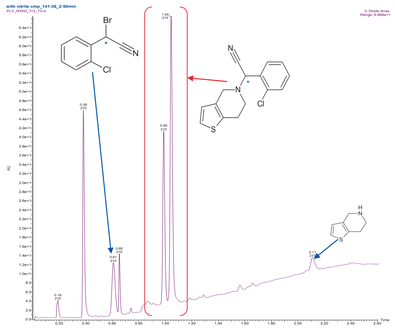
The second reaction step for the synthesis of clopidogrel utilizes a chiral starting material which is reacted with 4,5,6,7-tetrahydrothieno[3,2-c]pyridine to yield a chiral intermediate product (*below*). The chiral screening results for the chiral starting material indicated a Chiralpak ID column provided the best separation of the two enantiomers. It was hypothesized that the same chiral column would provide separation of the chiral product as well. Interestingly, the Chiralpak ID did not provide a separation of the enantiomers; however, the worst column choice from the initial screening results of the chiral starting material (Chiralpak IB) provided the best separation for the chiral intermediate generated from the second step of the clopidogrel synthetic route. Since the UPC² was coupled to MS, the peaks were easily mass confirmed (*spectra not shown*).



Chiralpak ID Results

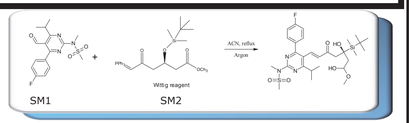


Chiralpak IB Results

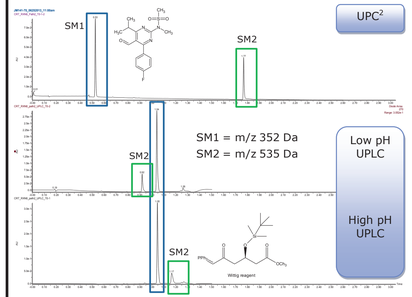


Justifying an Orthogonal Approach to Reversed-Phase LC (RPLC)

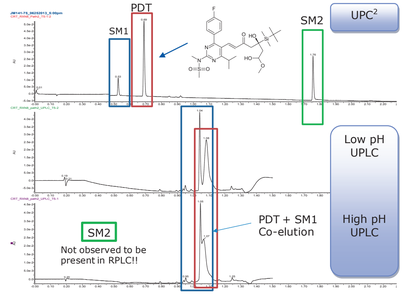
The eighth reaction step in the synthesis route for rosuvastatin involved the use of a Wittig reagent to aid the production of the desired enantiomer of the final product for rosuvastatin without use of a chiral resolving agent. The details of the reaction are described (*right*).



Time Point 0: Before reaction occurs



Time Point 5: 6 hours



It is common for a workflow to screen high and low pH mobile phases in an effort to promote selectivity for a given separation. The initial time point of this reaction was monitored with UPLC and UPC² (*above, left*). Our results show better separation of the starting materials when using UPC² when compared to the RPLC approach. Elution of SM2 is not easily resolved from SM1 for RPLC, regardless of pH. Additionally, UPC² provided better peak shape for SM2. Each of these improvements realized by UPC² aided in monitoring the reaction and quantitation of the yield and purity.

As the reaction progressed, aliquots were taken every hour. Shown (*above, right*) are the results for the UPC² and high/low pH LC chromatograms of an aliquot taken after 6 hours. The RPLC approach indicates SM2 has been fully consumed; however UPC² clearly shows its presence. More importantly, the separation of the product from SM1 was not optimal in either of the RPLC pH results and the product peak was quite broad. UPC² provided optimal resolution of all the constituents in the mixture. Because of this added resolution, purification of this material would be easier .

CONCLUSIONS

- **Mass spectrometry** was clearly needed to identify non-chromophore containing analytes which are typical at early stages of synthetic reactions
- The 'ease of use' column management allowed for the expanded **selectivity** exploration with a stationary phase agnostic capability. This can only be provided by the convergence chromatographic approach if performed on a single system
- **Chiral Screening** is a necessity throughout each step of the synthesis that contains enantiomeric analytes
- Having an **Orthogonal Approach** to RPLC can aid the monitoring and quantification of yield and purity as well as facilitate the purification decision process

Notable *Key* Instrument Capabilities

⇒ **Stationary Phase Agnostic**
A variety of columns such as RPLC, NPLC, HILIC, and chiral columns can be used without changing mobile phase, diluents, or instrumentation

⇒ **Independent Temperature Control**
Advantageous for achiral/chiral screening. Achiral experiments may be run at higher temperatures than the chiral experiments. The column manager allows for independent control of each column compartment maximizing the life of chiral columns which typically have upper temperature limitations of 40-45°C not in use during storage in the column manager.

⇒ **Direct Injection of Organic solvents**
All of the synthetic processes monitored consisted of organic aliquots of the reaction mixtures. With this technique, conversion to 'RPLC friendly' diluents was not required.

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