Development of an Online and Stop-flow LC/Benchtop-NMR

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Abstract

Complex mixtures are known to cause difficulties in structural elucidation in the NMR. While LC provides the ability to separate mixtures, most detectors do not provide the ability to determine information regarding the structure of the analyzed compounds. Previous iterations of LC-NMR systems involved large, high-field NMRs, making the coupling of the two systems both difficult and prohibitively expensive. The advent of more compact benchtop-NMR systems with higher field-strengths has allowed NMR to be more accessible as an LC detector while providing a reasonable level of sensitivity.

While online measurements require only high concentrations of analyte and continuous NMR scans, stop-flow LC minimizes this problem by holding the analyte in the NMR cell. However, this requires the calculation of the delay between the photodiode array detector and the NMR flow cell. Stop-flow allows a lower concentration of analyte to be identified, benefiting from the higher number averaged NMR scans typically performed. Mobile-phase solvents commonly used in LC have the potential to cause NMR analyte peak interferences, which add further complexities to compound identification. Initial findings in both online flow detection for higher concentration samples and stop-flow techniques for lower concentration analytes indicate the viability of this technique.

Objectives

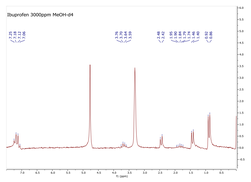
To develop viable methods for the connection of a liquid chromatograph with a benchtop-NMR system for identification and analysis in the laboratory and industrial environment in the following manner:

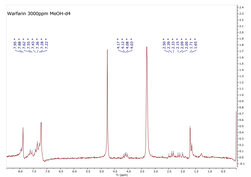
- Online detection using a low flow rate in the LC and going to the NMR.
- Stop-flow detection by correlating the peak appearance time in the PDA to the highest concentration of analyte into the NMR flow cell for analysis.
- Methods to incorporate conditions found in industrial processes that involved undeuterated solvents, such as in large scale reactors.

Instrumentation and Setup

- Shimadzu liquid chromatograph (LC-20) with Photodiode Array Detector (SPD-M20A)
- QMagnetics 125 MHz NMR
  - Connection from the LC to the NMR inlet port was tubing with an inner diameter of 0.13mm, with the NMR flow cell with an inner diameter of 1.00mm.

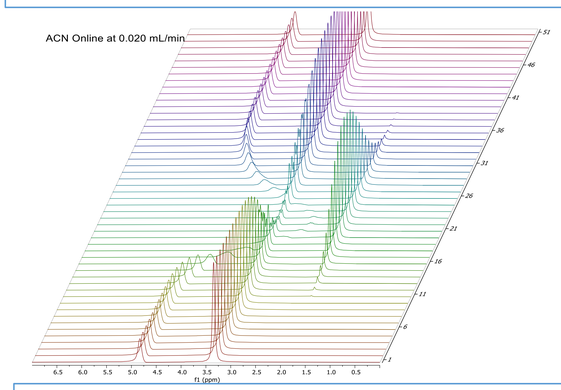
Deuterated Solvent NMR





Instrumentation and Setup

Initial online LC-NMR connection verification testing was conducted using a 50uL acetonitrile injection with an isocratic flow rate of 0.02mL/min methanol. Continual NMR scans were generated over time, with an overall scan time of 14 seconds as seen below.



- Appearance of the acetonitrile began at 9.10 minutes and reached maximum signal at 11.90 minutes based on the initialization time of the LC injection.
- This was repeated with a lower concentration of acetonitrile and similar maximum signal strength was observed, confirming the link between the two systems.
- As acetonitrile is not detectable in a PDA, other compounds were then tested.

Initial Samples

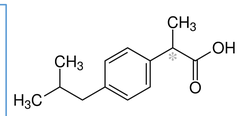
Four analytes were selected with notably different and distinct spectra patterns in NMR data. Initial benchtop-NMR scans were performed in deuterated and undeuterated methanol.

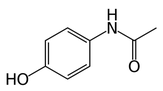
Ibuprofen
- Series of clear doublets and multiplets spread across the NMR spectrum

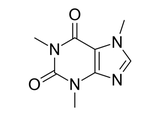
Acetaminophen
- Distinct, sharp peaks outside the methanol solvent peak in the NMR spectrum

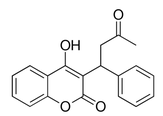
Caffeine
- Distinct, sharp peaks focused in the 3ppm-5ppm region, where methanol solvent peaks occur in the NMR spectrum

Warfarin
- Highly complex multiplet series, with varying levels of strength across the NMR spectrum









Online Testing and Stop-Flow Testing Method Comparisons

Online Testing:
- NMR scans required to be performed throughout the entire test
- Very low flow rates are needed to obtain resolvable data (0.010mL/min)
- Lower sensitivity
- Requires very high analyte concentration

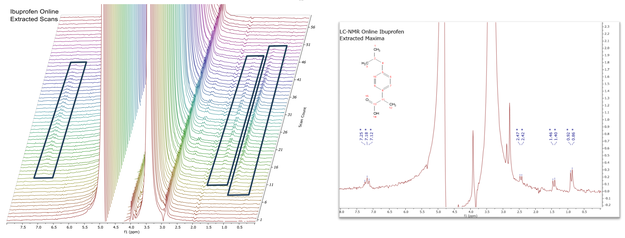
Stop-flow Testing
- NMR scans only need to be performed during the stop segments
- Faster flow rates can be performed but can require temperature stabilization of the NMR flow cell
- Higher sensitivity
- Requires lower analyte concentrations
- Times for flow stopping must be determined from an initial LC test and be correlated to prior NMR peak appearance or calculated transport time from the PDA to the NMR cell.
- Length of the stop time must be determined by the number of scans required as well as potential temperature stabilization of the NMR before initializing the NMR scans.

Online LC-NMR Results

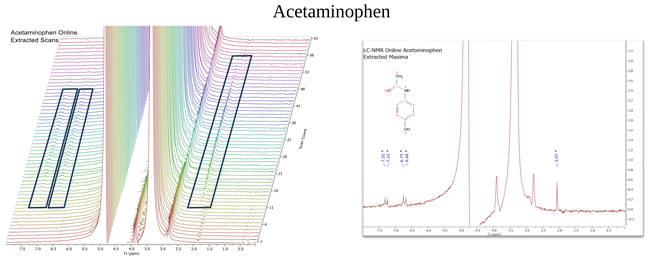
The four analytes were measured individually in undeuterated methanol, and the start time of the LC corresponded to the start of the NMR. Analysis was performed at 0.01mL/min to increase the resolution. Peak maxima based on the start time of the LC and of the peak appearance of the compound within the PDA.

These were repeatable across the compounds and the NMR peak maxima were seen at approximately the same times.

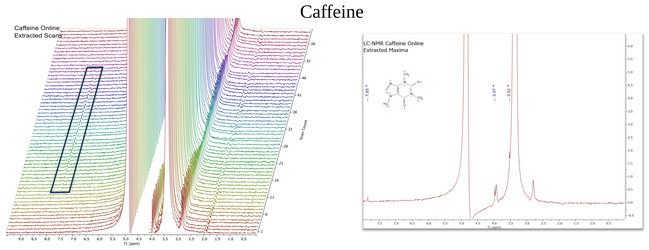
Ibuprofen



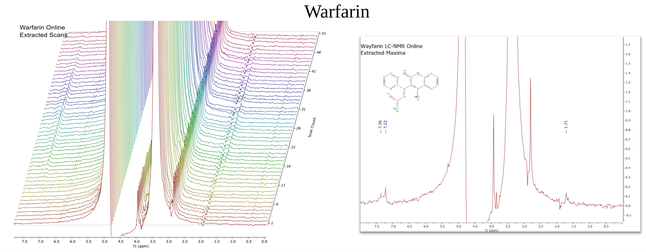
Acetaminophen



Caffeine



Warfarin



Stop-Flow Results

Using the data obtained from the online LC-NMR, a relationship between the PDA peak appearance times and the strongest NMR signal was determined. This maxima indicates the time of highest concentration in the NMR cell, allowing the calculation of the optimal stop-flow time for future tests with differing analyte retention times.

Each of the previous four compounds were then tested at designated stop-flow maxima times with significantly cleaner signal, due to the increased number of NMR scans.

Future Work

- Column separations and individual analysis of each analyte in the NMR
- Reduce the amount of analyte needed
  - Smaller amounts can be performed but due to detection limits of an NMR, a higher concentration must be used when compared to traditional LC testing.
  - One potential avenue is reduction of the inner diameter of the NMR flow cell tubing. Currently, a dilution effect of approximately 75% is observed. A decrease in the inner diameter could significantly reduce the dilution, resulting in a much lower concentration of sample to be injected into the LC.
- Determine the optimal flow rate of the LC versus the NMR cell temperature equilibration time
  - Higher flow rates from the LC are required for separation and reasonable test time, but as the flow rate increases the more unstable the temperature in the NMR becomes. A relationship must be determined to compensate for the required equilibration time before initializing the NMR scan sequence.

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