

Real-Time Reaction Monitoring with Micro-Scale HPLC

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Abstract

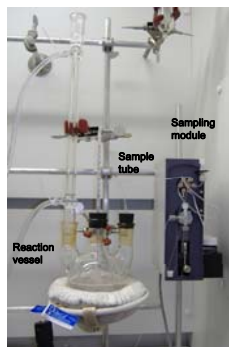
The ability to monitor impurity and by-product formation during a chemical synthesis provides information critical for minimizing or reducing waste products. Additionally, continuous determination of conversion, % impurity, as well as enantiomeric excess during a chiral chemical synthesis provides information critical for synthesis optimization and development. However, automated real-time reaction sampling combined with chromatographic analysis is not routinely performed for the purpose of process development and optimization. A primary reason has been the lack of an integrated sampling/analysis platform. An innovative sampling module design has now been integrated with a micro-scale HPLC platform to provide a mobile analysis system that utilizes the advantages of micro-scale chromatography to provide automated real-time analytical information. Micro-scale HPLC generates high-quality analytical information, equivalent or superior to conventional HPLC instruments while providing greater than 90% savings in solvent usage and generation of hazardous solvent waste. The analyzer's close proximity to the reaction and the inherently low-volume sample requirements of micro-scale HPLC reduce the sampling volume to only 20 μ L per data point. The system allows for unattended continuous analysis during extended chemical syntheses. Reaction monitoring data will be presented for both overnight and multi-day syntheses showing a significant reduction in solvent usage and the ability to monitor reaction byproducts. Conversion and enantiomeric excess data was collected on-the-fly for the several these reactions.

Integrated Sampling and Analysis On-The-Fly

The ExpressRT-100 provides fully automated and integrated reaction sampling and liquid chromatographic analysis. Currently, to monitor a reaction, aliquots of a reaction are manually drawn and then submitted for analysis. Sample submissions could be delayed by analyst priorities or analytical lab backlogs. On-line analysis until now has been limited to the use of FTIR probes. Using the integrated sampling module and HPLC analysis, real-time information on the progress of chemical syntheses are available for process development and reaction monitoring. Integrated user-friendly software performs rate, conversion and enantiomeric excess (ee) analysis in real-time. With potential cycle times of 3 minutes, tens to hundreds of data points can be acquired over the span of hours or days. The micro-scale HPLC analysis module provides quick, real-time separations with high efficiency. The low-solvent demands for this system require only a few milliliters of mobile phase to analyze over 100 samples.



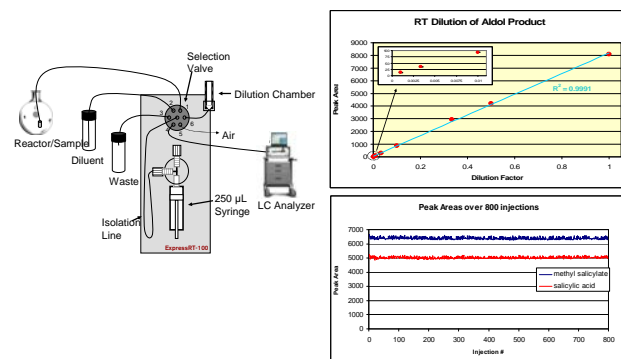
The ExpressRT-100 for real-time automated reaction monitoring.



Setup for reaction monitoring. Sampling module is affixed to lab frame adjacent to vessel. Six-port valve with syringe pump aspirates sample from the vessel through a filter. Dilution is performed in a mixing chamber and sample delivered to LC through a low-volume capillary.

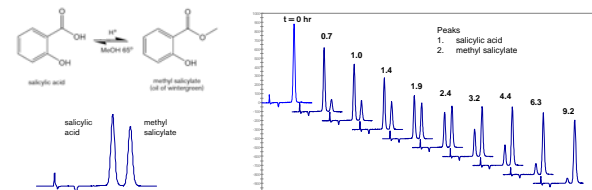
Automated on-line sampling and dilution

A schematic illustration of the sampling module used in the ExpressRT is shown below. The sampling module can be used for sample quenching and/or on-line dilution. The figure below shows the excellent linearity of over three orders of magnitude for the on-line dilution. Three concentrations of acetophenone in 80:20 ACN:H₂O were used for the dilutions (10 μ L/mL for 0.001x and 0.003x dilution; 1 μ L/mL for 0.01x and .03x dilution; 0.1 μ L/mL for 0.1x, 0.3x and 1x dilutions). Peak areas were scaled to account for the three starting concentrations. A mixture of 5 mg/mL carbon black (<0.06 μ m), salicylic acid and methyl salicylate in methanol was used to test robustness. Data from over 800 samples taken from this heterogeneous mixture are shown below.



Esterification of Salicylic Acid

The esterification of salicylic acid to methyl salicylate was monitored with automated sampling using the ExpressRT system. 10 g of salicylic acid was dissolved in methanol to which 5 mL of concentrated sulfuric acid was added. The reaction was monitored over 12 hours. Both the salicylic acid reactant and the methyl salicylate product were separated by LC and monitored by UV absorption.

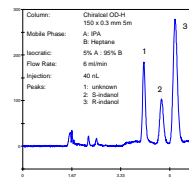
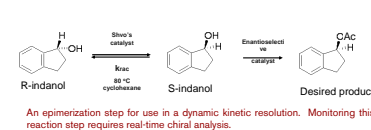


Reaction of salicylic acid to methyl salicylate (top). Sample chromatogram showing the resolution of the reactant and product of the reaction (bottom).

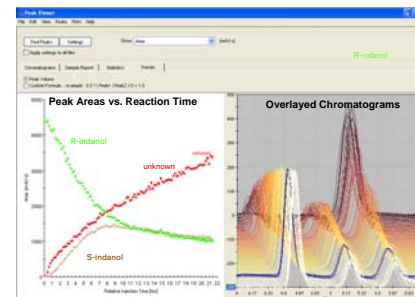
Chromatograms from periodic sample of the reaction described showing the evolution of the reaction from reactants to products. Selected time points during the 12 hour reaction are shown.

Real-Time Chiral Analysis with PeakViewer software

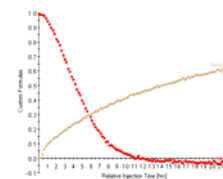
The automated sampling, dilution and fast chromatography of the ExpressRT was used to monitor the racemization of R-indanol. Increasing racemization rates (k_{rac}) through the use of catalysts is critical in chiral synthesis. In the example below, only S-indanol in a 50:50 racemic mixture is converted to the desired product. Use of Shvo's catalyst can double product yield as it allows conversion of R-indanol as well. A 2% molar ratio of Shvo's catalyst and 420 mg of R-indanol were added to 210 mL of cyclohexane. The reaction was monitored over 22 hours.



Sample diluted and injected for normal phase chiral chromatography analysis.



R-indanol, Unknown side product and S-indanol peak areas updated and graphed in real time. All chromatograms from 22 hour reaction overlaid and offset for display.



Enantiomeric excess (ee) and impurity fractions as calculated and graphed by PeakViewer software.

Conclusion

The automated sampling, dilution and fast chromatography of the ExpressRT-100 make it well-suited for monitoring reactions. Even analytically-challenging chiral reactions can be monitored. Valuable information such as percent conversion, enantiomeric excess and formation of impurities can be displayed in real time and allow process optimization on the fly. Analytical data on a range of reactions were collected without the need for human attendance.

Acknowledgements

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1. Wes A. Schafar, Steve Hobbs, Jason Rehm, David Rakestraw, Charles Orella, Mark McLaughlin, Zhong Ge, and Christopher J. Welch *Org. Process Res. Dev.*, **2007**, *11*, (5), pp 870-876.