

Real-Time Chiral Reaction Monitoring with Micro-Scale HPLC

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Abstract

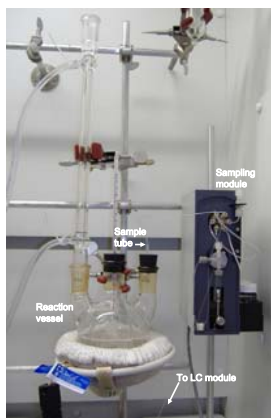
Real time automated sampling coupled to chromatographic analysis for chiral process development and optimization is not routinely performed. The ability to determine conversion, % impurity, as well as enantiomeric excess continuously during a chiral chemical synthesis provides information critical for optimization and development. We have developed an integrated sampling and analysis platform that utilizes the advantages of micro-scale chromatography to provide automated real-time analytical information. This mobile platform can be wheeled into any laboratory and up to the chemical hood where the sampling module is affixed to a conventional lab frame adjacent to the reaction vessel. This proximity to the reaction and the inherent low-volume requirements of micro-scale HPLC reduce the sampling volume to 20 μ l per data point. The use of this system allows for unattended continuous analysis of overnight or multi-day syntheses. Using the ExpressRT-100 "Reaction Tracker", conversion and enantiomeric excess (ee) data was collected on-the-fly for an esterification reaction and the asymmetric reduction of a ketone.

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. Overnight data points require human attendance. Sample submissions could be delayed by analyst priorities or analytical lab backlogs. On-line analysis information may be limited to FTIR. 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 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 separations with high efficiency. The low-solvent demands for this system require only a few milliliters of mobile phase to analyze over 100 samples.



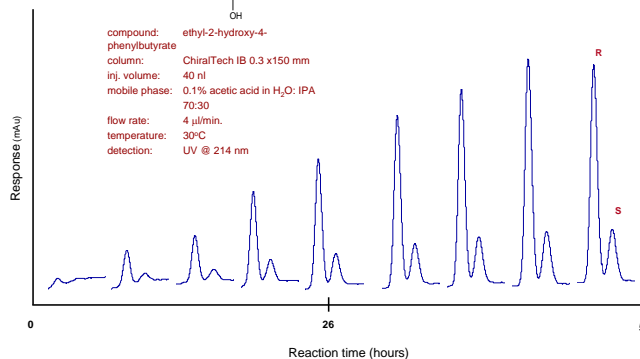
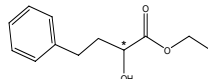
The ExpressRT-100 for real-time automated reaction monitoring.



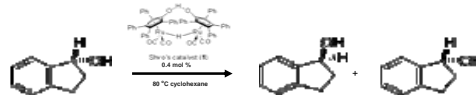
Set-up for reaction monitoring. Sampling module is affixed to lab frame adjacent to vessel. Six-port valve with syringe pump aspirates sample from the vessel through a filter. Dilution is performed in a mixing chamber and sample delivered to LC through a low-volume capillary.

Enantiomeric Excess from a Biocatalytic Reaction

The asymmetric reduction of a ketone was monitored over several days. In an 500 mL Erlenmeyer flask, alcohol dehydrogenase (Codexis, Redwood City, CA, cat # 03.11) was combined with ethyl-2-oxo-4-phenylbutyrate and a NADP co-factor in 100 mL of a 30:70 mixture of IPA and 0.1M Phosphate buffer (pH7). The sampling tube with stainless steel filter assembly was inserted into the stirring reaction broth. The enzyme selectively reduced the ketone to yield two enantiomers of ethyl-2-hydroxy-4-phenylbutyrate. Using a chiral separation method, the enantiomeric excess (ee) and product areas were monitored for over 50 hours. Sampling and analysis was done automatically every 50 minutes over the three days the reaction was running. Equilibrium was reached in approximately 48 hours. The enzyme was found to be selective for the R enantiomer (ee = 59%). Once both enantiomers were above the LOQ for the analyzer, the ee was seen to remain constant as conversion increased.

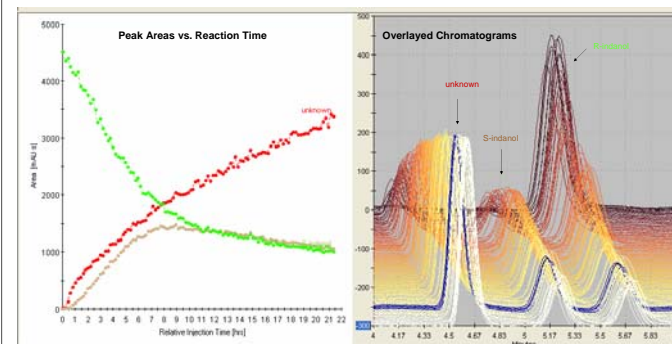


Racemization of R-Indanol



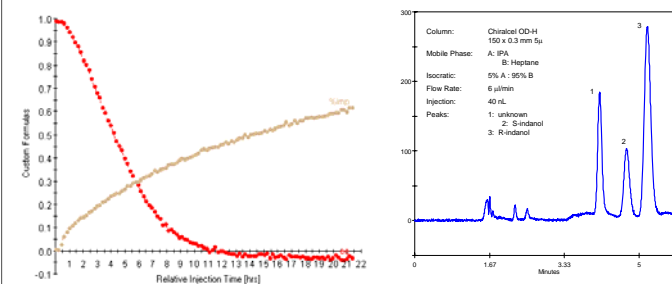
An epimerization step for use in a dynamic kinetic resolution. Monitoring this reaction step requires real-time chiral analysis.

Real-Time Chiral Analysis with PeakViewer software



Unknown side product, S-Indanol and R-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 as a fraction calculated and graphed by PeakViewer software.

Sample diluted and injected for normal phase chiral chromatography analysis.

Conclusion

Collecting real-time information during chiral reactions was accomplished by the ExpressRT-100 "Reaction Tracker". Aliquots of a biocatalytic and racemization reaction were automatically sampled and analyzed for conversion and enantiomeric excess (ee). In addition side product and impurity information was obtained in real-time. Automated and integrated sampler, analyzer and software allow for process optimization on the fly. Analytical data on a multi-day reaction was collected automatically, without the need for human attendance.