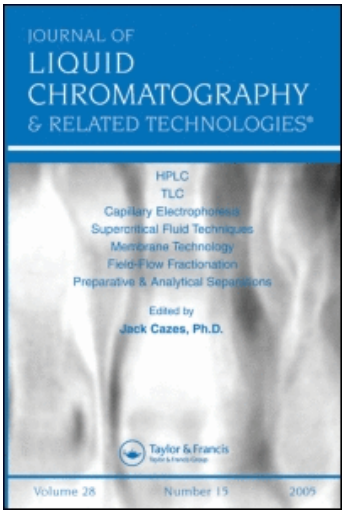

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Rapid Analytical Method Development Using Multiparallel Microfluidic High-Performance Liquid Chromatography in Support of Pharmaceutical Process Research

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Abstract: Rapid chiral method development using microfluidic multiparallel HPLC is illustrated with several examples of 'real world' pharmaceutical intermediates for which speed was critical for successful process development. It is shown that this rapid chiral method development approach is suitable for support of high throughput screening of enantioselective catalytic hydrogenation reactions and other high throughput experimentation initiatives in pharmaceutical process research.

Keywords: Asymmetric hydrogenation, Catalyst screening, Chromatography, High throughput, HPLC, Microfluidic, Multiparallel, Normal phase

INTRODUCTION

In a previous study we reported the use of a microfluidic multiparallel HPLC system for the rapid development of methods for the chromatographic separation of enantiomers.[1] In that study we

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examined several model compounds that are commonly employed to test the separation performance of chiral columns, demonstrating an ability to rapidly develop fast methods for enantiopurity determination. In the present study we demonstrate the advantages of the multiparallel format for method development and high throughput analysis to support high throughput experimentation initiatives in pharmaceutical process research. In these studies, 'real world' process research samples from high throughput catalysis investigations are presented to illustrate the strength and versatility of the new approach. High-throughput experimentation initiatives requiring rapid enantiopurity determination have become a topic of great interest over the past few years for screening enantioselective catalysts, enzymes, or separation methods.[2–12] The approach described herein shows promise as a potential general solution to this problem.

EXPERIMENTAL

HPLC Instrumentation

An Eksigent Express-800 parallel microfluidic HPLC system was used in the study (Eksigent Technologies, LLC, Dublin, CA, USA).[13] The system is equipped with eight HPLC channels that can be operated simultaneously and independently. It comes with a dual head CTC LEAP auto-sampler, allowing injection from autosampler vials and 96 or 384-well microplates. Individual diode array UV detectors and two pumps are used for each channel. Thus different flow rates, gradients or eluents can be run concurrently on different channels, and different UV wavelengths.

Chemicals

Ethanol, 2-propanol and heptane were obtained from EM Science (Gibbstown, NJ, USA). Sample mixtures and reference standards were obtained from the catalysis screening group within Merck Process Research (Merck Research Laboratories, Rahway, NJ, USA).

HPLC Columns

Chiralpak AD-H, Chiralpak AS-H, Chiralcel OD-H and Chiralcel OJ-H columns were used. The columns (0.3×150 mm) were obtained from Chiral Technologies (West Chester, PA, USA).

RESULTS AND DISCUSSION

Asymmetric catalytic hydrogenation is a preferred technique to economically prepare enantiopure pharmaceutical intermediates with high yield and enantiopurity. Identification of suitable hydrogenation conditions typically involves the investigation of numerous ligands, metal precursors, solvents, additives, temperatures and pressures. With so many possible combinations, initial screening for asymmetric hydrogenation conditions can involve the preparation of literally hundreds of different samples, each of which must be monitored for extent of conversion to the desired enantiomer of the product compound.[1,14–18] Not surprisingly, rapid analysis of enantiopurity has become a paramount concern and essential tool for effective exploration of conditions for asymmetric hydrogenation. In addition, rapid method development is also very important in this context, because without a suitable analytical method, enantiopurity cannot be assessed quickly. Thus, in order for analysis to avoid becoming a 'bottleneck' in the overall workflow, it is very important to rapidly develop fast methods which are then utilized for high throughput enantiopurity analysis. Our previously described multiparallel chromatographic approaches for both the method development and the high throughput analysis[14] are proving to be quite useful, as is illustrated here with several recent examples from these laboratories.

The general strategy for method development is illustrated with an example presented in Figure 1. In this example a fast analytical method was needed to support catalysis screening for the asymmetric hydrogenation reaction depicted in Figure 1(a). Initially, column screening was performed to find an appropriate chiral stationary phase for the separation using the previously described approach in which the eight independent channels of a multiparallel HPLC instrument are arranged to rapidly evaluate 4 different chiral stationary phases (CSPs) and two different mobile phase combinations. While a number of variations on this general theme are possible, simply screening four carbohydrate-derived CSPs (Chiralpak AD-H, Chiralpak AS-H, Chiralcel OD-H, Chiralcel OJ-H) and two different mobile phase gradients (ethanol/heptane and 2-propanol/heptane) leads to the identification of a suitable analytical method in many instances. A universal gradient approach is generally employed for initial chiral method development screening. Again, variations are possible, but a gradient of 5% alcohol modifier in heptane for 4 min, followed by a ramp to 40% modifier over 18 min, followed by a 3 min hold and 5 minutes reequilibration time was found to be generally useful. The total run time (for 1 sample with 8 columns), including equilibration before the injection, was thus only 30 min (less than 4 minutes per chromatogram)

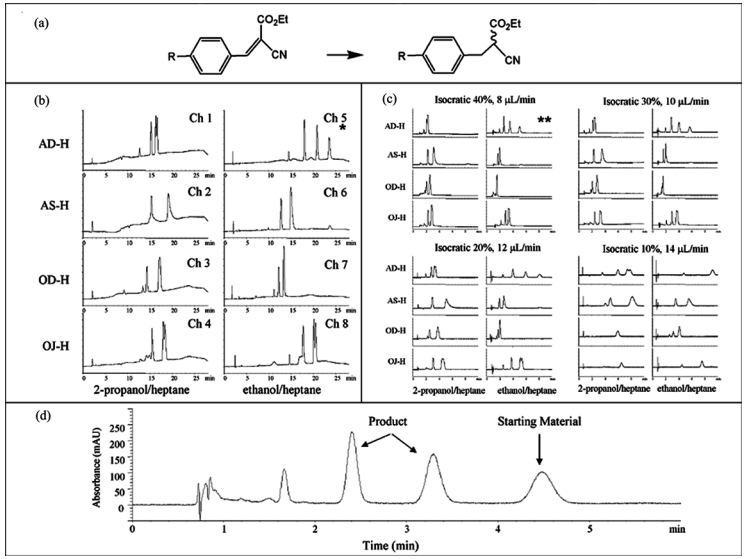

Figure 1. Multiparallel approach to chiral HPLC method development for high throughput screening of asymmetric hydrogenation (a) Reaction scheme for the introduction of a stereogenic center into a pharmaceutical intermediate *via* asymmetric hydrogenation. (b) Chiral method development using a standard gradient elution approach with four different columns and two different eluents. Screening results are obtained within 30 min. Conditions: 5-min equilibration, 5% alcohol modifier in heptane for 4 min, followed by a ramp to 40% modifier over 18 min, followed by a 3-min hold; flow rate 4 μL/min; detection at UV 210 nm; 0.3 mm i.d. × 150 mm columns, injection size 40 nL. (c) Automated isocratic method optimization. Alcohol modifier (2-propanol or ethanol) concentrations in heptane: 40%, 30%, 20% and 10% (v/v). (d) Optimized chiral HPLC method for high throughput analysis. Chiralpak AD-H, 40% ethanol/heptane, 12 μL/min, 20 nL injection volume.

greatly reducing the time for method development screening relative to an approach using sequential column switching.

The results of initial method development screening are illustrated in Figure 1(b), where it can clearly be seen that Chiralpak AD-H with an ethanol/heptane eluent easily resolves the two enantiomers of product from the starting material. Method optimization is next carried out to afford the fastest possible method for carrying out high throughput analysis. The total number of samples to be analyzed dictates to some degree the amount of method optimization that is needed, with the initial screening method even being suitable if only a few samples are to be analyzed. For high throughput analysis needs, translation to a fast isocratic method is generally preferred. Method optimization

on the multi-parallel system is carried out by running all 8 columns simultaneously in isocratic elution mode, with varying alcohol modifier (2-propanol or ethanol) concentrations in heptane, e.g., 40%, 30%, 20% and 10% (v/v), as illustrated in Figure 1(c). In this example, flow rate was adjusted for each mobile phase concentration so as to afford comparable back pressures for each eluent composition. By using this approach, the run time for the optimization experiments is kept to a minimum. It is noteworthy that with a multiparallel approach all 8 channels are used for the optimization, as opposed to the sequential approach, where the need to conduct optimization studies in a sequential fashion means that typically, only the best candidate separation is performed. We have recently shown that multiparallel optimization studies can be advantageous in some situations where second or even third choice screening hits sometimes end up giving rise to the best high throughput analysis method.[11] This is especially true for multi-component separations.

From these initial isocratic optimization experiments a suitable method can often be selected and used without further adjustments, or the method can be fine tuned to give an even shorter runtime if this is needed (Figure 1(d)). It is noteworthy that the complete analytical method development (i.e., column screening and optimization) is accomplished within only 2 h using the multiparallel approach.

In the second example, a method was needed to support screening of an enantioselective reductive amination, in which a prochiral ketone was converted, *via* an imine intermediate, to an enantioenriched amine (Figure 2). A variety of catalysts and conditions were investigated to find suitable conditions for conversion and selectivity for the imine reduction. As unreacted imine reverted to ketone in some samples, a suitable analytical assay that resolved the enantiomers of product amine from both imine and ketone was needed.

Method development was carried out in the same fashion as described in the first example. The initial gradient screen for a mixture of the four components, ketone, imine and the two enantiomers of the chiral amine is shown in Figure 2(b). It can be seen that one combination of conditions results in a good separation of all four components – Chiralcel OJ-H with heptane/ethanol (channel 8). The column screening procedure took only 30 minutes as the columns were run in parallel. The results of the screen were quickly optimized to give a fast method for analysis that separates all four components, the ketone, imine and the two enantiomers, of the chiral amine. The optimized method is shown in Figure 2(c). As seen from the Figure, all components are well separated in a chromatographic method with a relatively fast run time of about 6 minutes.

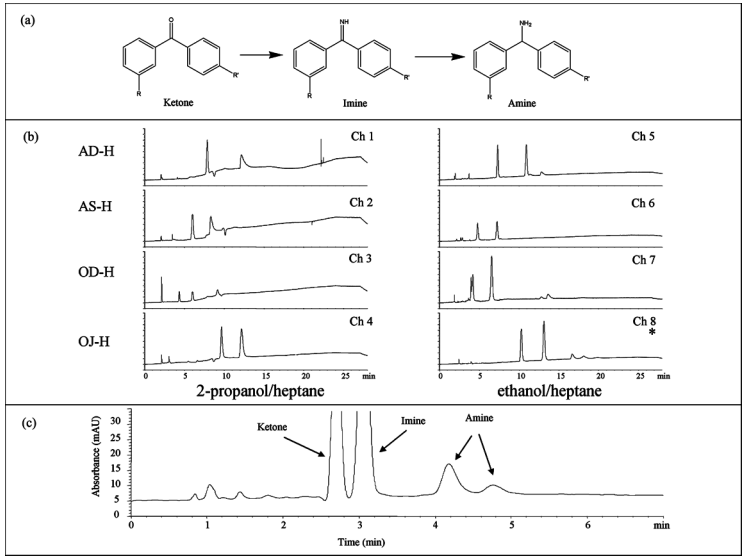

Figure 2. Multiparallel chiral HPLC method development for monitoring of the conversion of a prochiral ketone, *via* an imine intermediate, to an enantioenriched amine. (a) Reaction scheme. (b) Multiparallel chiral HPLC method development screening. (c) Optimized separation. Chiralpak OJ-H 15 × 0.03 cm, 20% ethanol/heptane, 12 mL/min, 20 nL injection volume, detection at UV 210 nm.

Several additional examples of the approach are illustrated in Figure 3. In the example presented in Figure 3(a), a method was required to support the study of the racemization of a bis-aryl substituted chiral amine. Fast methods are often important for studying racemization, not only to enable high throughput screening of reaction conditions, but also to allow timecourse monitoring of racemization reactions with reasonably short sampling intervals. Method development was again carried out using the multiparallel approach to afford a suitable method for chiral analysis, with resolution of an *N*-methyl pyrrolidone solvent peak from the two enantiomers of the compound of interest within 3 min.

In the example presented in Figure 3(b), a method was required to monitor the generation of an enantioenriched secondary alcohol from the corresponding α-ketoamide. In this example, the multiparallel method development approach was used to generate a 6 minute method that resolved not only the two product enantiomers and starting material, but also two unidentified degradation products.

Finally, the example presented in Figure 3(c) illustrates a method that was developed to resolve the enantiomers of a Boc sulfonamide

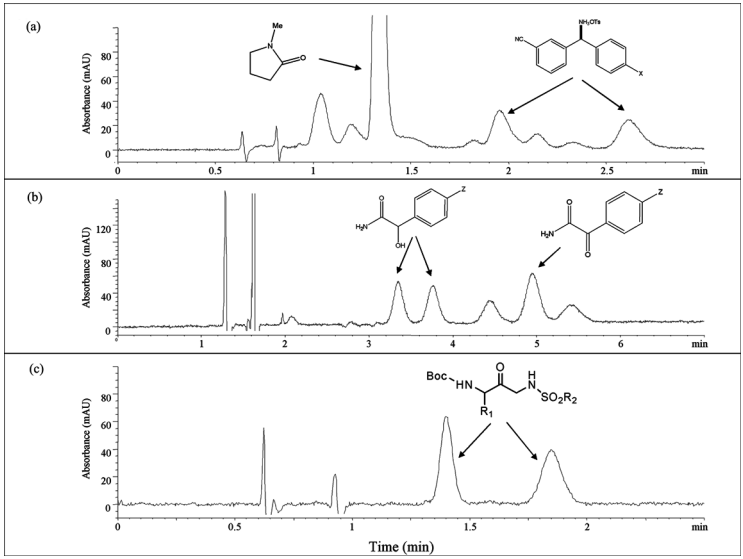

Figure 3. Examples of analytical methods that were developed using multiparallel microfluidic screening and isocratic method development. (a) Method to monitor the racemization of a bis-aryl amine substituted chiral amine. Conditions: Chiralpak AD-H 15×0.03 cm, 20% ethanol/heptane, 12 μL/min, injection size 20 nL; detection at UV 210 nm. (b) Method for the monitoring of the generation of a chiral secondary alcohol from the α-ketoamide. Conditions: Chiralcel OD-H 15×0.03 cm, 20% ethanol/heptane, 6 μL/min, injection size 20 nL; detection at UV 210 nm. (c) Method for the evaluation of the enantiomeric excess of a Boc sulfonamide intermediate. Conditions: Chiralpak AD-H 15×0.03 cm, 10% ethanol/heptane, 14 μL/min flow rate; detection at UV 210 nm; 0.3 mm i.d. × 150 mm columns, injection size 20 nL; detection at UV 210 nm.

intermediate, with a chromatographic run time of about 2 min being possible in this instance.

CONCLUSION

The 'real world' examples presented in this paper illustrate the suitability of the microfluidic multiparallel HPLC system for rapid development of chiral assays to support pharmaceutical process research. These examples from actual process development studies carried out in these laboratories demonstrate the strength of this new approach for enabling high throughput experimentation studies, and suggest that multiparallel

microfluidic HPLC may become an important tool for enabling high throughput experimentation to support pharmaceutical process research.

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REFERENCES

1. Sajonz, P.; Schafer, W.; Gong, X.; Leonard, W.R.; Biba, M.; Welch, C.J. Multiparallel chiral method development screening using an 8-channel microfluidic HPLC system. Chirality **2006**, *18*, 803–813.
2. Finn, M.G. Emerging methods for the rapid determination of enantiomeric excess. Chirality **2002**, *14*, 534–540.
3. Nelson, T.D.; Welch, C.J.; Rosen, J.D.; Smitrovich, J.H.; Huffman, M.A.; McNamara, J.M.; Mathre, D.J. Effective use of preparative chiral HPLC in a preclinical drug synthesis. Chirality **2004**, *16*, 609–613.
4. Welch, C.J.; Albaneze-Walker, J.; Leonard, W.R.; Biba, M.; DaSilva, J.; Henderson, D.; Laing, B.; Mathre, D.J.; Spencer, S.; Bu, X.; Wang, T. Adsorbent screening for metal impurity removal in pharmaceutical process research. Org. Process Res. Dev. **2005**, *9*, 198–205.
5. Reetz, M.T.; Kuhling, K.M.; Hinrichs, H.; Deege, A. Circular dichroism as a detection method in the screening of enantioselective catalysts. Chirality **2000**, *12*, 479–482.
6. Welch, C.J.; Grau, B.; Moore, J.; Mathre, D.J. Use of chiral HPLC-MS for rapid evaluation of the yeast-mediated enantioselective bioreduction of a diaryl ketone. J. Org. Chem. **2001**, *66*, 6836–6837.
7. Tao, W.A.; Cooks, R.G. Chiral analysis by MS. Anal. Chem. **2003**, *75*, 25A–31A.
8. Schug, K.A.; Maier, N.M.; Lindner, W. Chiral recognition mass spectrometry: remarkable effects observed from the relative ion abundances of ternary diastereomeric complexes using electrospray ionization. Chem. Commun. **2006**, *4*, 414–416.
9. Brewer, B.N.; Zu, C.; Koscho, M.E. Determination of enantiomeric composition by negative-ion electrospray ionization-mass spectrometry using deprotonated *N*-(3,5-dinitrobenzoyl)amino acids as chiral selectors. Chirality **2005**, *17*, 456–463.
10. Reetz, M.T. Directed evolution of an enantioselective enzyme through combinatorial multiple-cassette mutagenesis. Angew. Chem Int. Ed. **2001**, *40*, 3589–3591.
11. Pollard, D.J. Effective synthesis of (*S*)-3,5-bistrifluoromethylphenyl ethanol by asymmetric enzymatic reduction. Tet. Assym. **2006**, *17*, 554–559.
12. Truppo M.; Pollard D.J; Devine P. Enzyme-catalyzed enantioselective diaryl ketone reductions. Org. Letts. **2007**, *9*, 335–338.

13. www.eksigent.com
14. Sajonz, P.; Schafer, W.; Gong, X.; Shultz, S.; Rosner T.; Welch, C.J. Multiparallel microfluidic high-performance liquid chromatography for high-throughput normal-phase chiral analysis. J. Chromatogr. A **2007**, *1145*(1–2), 149–154.
15. Cai, C.; Chung, J.Y.L.; McWilliams, J.C.; Sun, Y.; Shultz, C.S.; Palucki, M. From high-throughput catalyst screening to reaction optimization: detailed investigation of regioselective suzuki coupling of 1,6-Naphthyridone Dichloride. Organic Process Research & Development **2007**, *11*(3), 328–335.
16. Tellers, D.M.; Bio, M.; Song, Z.J.; McWilliams, J.C.; Sun, Y. Enantioselective hydrogenation of an α-alkoxy substituted ketone with chiral ruthenium (phosphinoferrocenyl)oxazoline complexes. Tetrahedron: Asymmetry **2006**, *17*(4), 550–553.
17. McWilliams, J.C.; Sidler, D.R.; Sun, Y.; Mathre, D.J. Applying statistical design of experiments and automation to the rapid optimization of metal-catalyzed processes in process development. JALA **2005**, *10*(6), 394–407.
18. Hsiao, Y.; Rivera, N.R.; Rosner, T.; Krska, S.W.; Njolito, E.; Wang, F.; Sun, Y.; Armstrong, J.D.; Grabowski, E.J.J.; Tillyer, R.D.; Spindler, F.; Malan, C. Highly efficient synthesis of β-Amino Acid Derivatives via Asymmetric Hydrogenation of Unprotected Enamines. Journal of the American Chemical Society **2004**, *126*(32), 9918–9919.

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