Use of Chiral HPLC-MS for Rapid Evaluation of the Yeast-Mediated Enantioselective Bioreduction of a Diaryl Ketone

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Introduction

Enantioselective reduction of diaryl ketones can present a formidable challenge, even with the extensive selection of reagents and catalysts now available.[1] Yeast-mediated enantioselective bioreduction can be useful in such challenging cases, although identifying a yeast strain with suitable levels of enantioselectivity and reactivity for use in a bioprocess may require the evaluation of hundreds or thousands of different cultures.[2] A recurring problem in the evaluation of the of aryl ketone bioreductions by chiral HPLC with UV detection stems from the fact that residual starting ketone may complicate the determination of product alcohol enantiopurity. The greater molar absorbance of the ketone and the need to avoid peak overlap can result in excessively long assay times that make screening large numbers of cultures problematic. In this study we illustrate how chiral HPLC-MS can be used to allow overlapping ketone and alcohol peaks to be conveniently resolved by mass, allowing the rapid enantiopurity analysis required for high throughput reaction screening.

Results and Discussion

One of the principal challenges in converting an existing chiral HPLC method to MS detection can be the introduction of an ionization buffer to facilitate MS detection.[3] Placing buffers into the eluent entering the column can dramatically influence chromatographic performance and may shorten column lifetime. Significant effort is sometimes required to develop a method that provides both rapid enantioseparation and good MS ionization. Postcolumn addition of the ionization buffer may allow the user to couple an existing normal-phase chiral HPLC method with a known LC-MS ionization buffer, providing a workable chiral HPLC-MS method that requires only minor adjustments to optimize.

In the present instance we knew that the enantiomers of the alcohol product were easily separated by chiral HPLC on the Chiralpak AD column with ethanol/hexane mobile phases. Problems with ketone coelution made it difficult to resolve both enantiomers from the starting material, even with run times approaching 1 h. Automated screening using a dedicated robotic LC-MS instrument quickly revealed that both ketone and alcohol were easily ionized and detected by MS using a mobile phase containing ammonium formate/formic acid buffer at pH 3.5. Postcolumn addition of the ionization buffer was accomplished by inserting a 'T' into the line connecting the UV and MS detectors, into which was pumped a 2 mM solution of the ionization buffer in ethanol. We found that a flow rate of 1 mL/min for the HPLC pump and 0.5 mL/min for the ionization buffer pump provided good

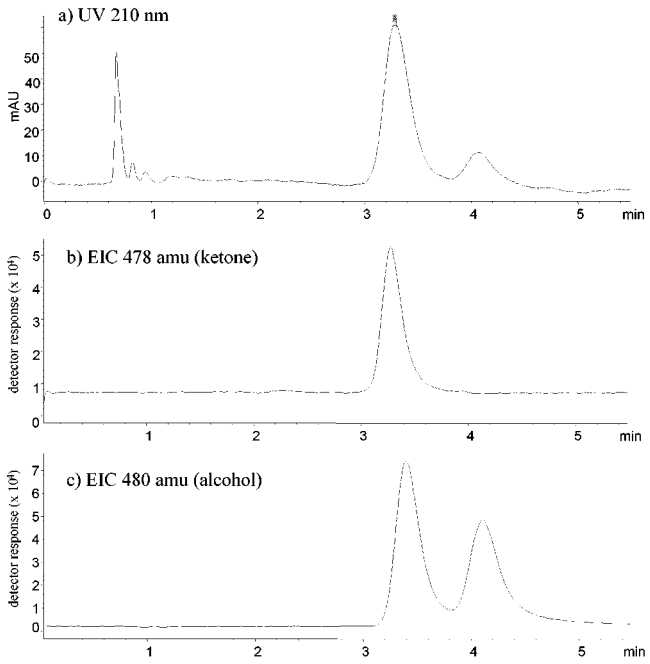

Figure 1. Chiral HPLC-MS with postcolumn addition of ionization buffer allows resolution of overlapping ketone and alcohol signals. Conditions: Chiralpak AD (4.6 × 50 mm); 3% EtOH/hexane; 1 mL/min with postcolumn addition of 2 mM ammonium formate/formic acid, pH 3.5 in ethanol at 0.5 mL/min; ESI MS detection, positive ion mode; SIM 478 amu (ketone) and 480 amu (alcohol).

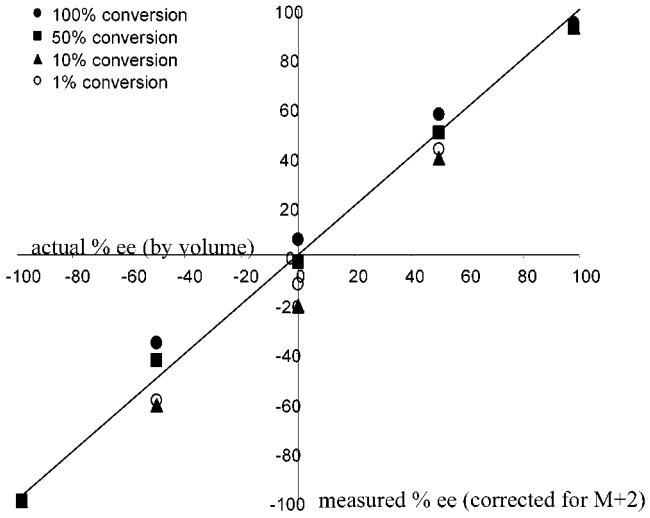

Figure 2. Alcohol enantiopurity can be reliably determined in the presence of unreacted ketone, especially at high % ee.

(1) Itsuno, S. *Org. React.* **1998**, *52*, 395−576.
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(3) Bakhtiar, R.; Ramos, L.; Tse, F. L. S. *Chirality* **2001**, *13*, 63−74.

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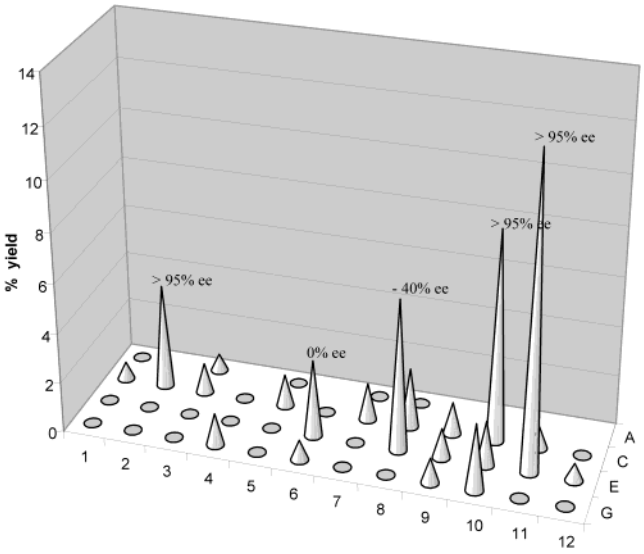

Figure 3. Results of a screen using a focused library of organisms previously shown to exhibit high enantioselectivity in the bioreduction of aryl ketones. Positive enantioenrichment is defined as enrichment in the desired second eluted enantiomer.

results. Using this approach, a 5-min assay that revealed both the extent and the enantioselectivity of ketone bioreduction was developed (Figure 1).

Accurate quantitation of overlapping peaks by LC-MS is sometimes thwarted by the influence of one compound on the ionization of another, a situation that is easily diagnosed using standards of known composition. Using pure solutions of ketone and the two alcohol enantiomers obtained by preparative chiral HPLC we obtained enantiopurity measurements which were suitable for initial screening of yeast cultures (Figure 2). Several cultures that formed the desired alcohol enantiomer with greater than 95% ee were identified, including selected strains of the organisms, *Hansenula nonfermentans*, *Rhodosporidium toruloides*, and *Candida bombi*, *santamariae*, *ernobii*, and *sorbophila* (Figure 3).

Conclusion

The use of chiral HPLC-MS with postcolumn addition of ionization buffer can be a useful and relatively easy to use tool for monitoring enantioselective reactions. This approach can be especially useful when unreacted starting material complicates traditional chromatographic analysis. As the influence of one compound on the ionization of another is possible, some care must be taken to ensure accurate quantitation of overlapping peaks. In this example, the use of a rapid chiral HPLC-MS reaction screening assay resulted in a substantial time savings.

Experimental Section

General Methods. Chiral HPLC-MS analysis was carried out using an Agilent HP 1100 MSD instrument fitted with a well plate autosampler and a Chiralpak AD column (4.6 × 50 mm; Chiral Technologies, Inc.). A mobile phase of 3% EtOH/hexane was passed through the column at 1 mL/min, and a solution of 2 mM ammonium formate/formic acid buffer at pH 3.5 in ethanol added via a connecting 'T' placed just before the mass spec detector was added at a flow rate of 0.5 mL/min. The mass spec detector was operated in the electrospray ionization mode, with positive ion detection and selective ion monitoring at 478 amu (ketone) and 480 amu (alcohol). Settings: Frag = 100; Vcap = 5000; drying gas flow = 13 L/min; nebulizer pressure = 60 psig; drying gas temperature = 350 °C.

Evaluation of Yeast Cultures. A 500 μL amount of active yeast culture solutions was placed into the individual wells of sterile 96 deep well plates, and ketone was added to a final concentration of 1 mM. The well plates were incubated with shaking at 29 °C for 1 day, and then 1 mL of ethyl acetate was added to each well. The plates were agitated and, after settling, 600 μL of the upper organic phase from each well was transferred into the wells of fresh plates, which were then evaporated to dryness. A 300 μL amount of 3% ethyl acetate in hexane solution was then added to each well, and the plates were placed into a microplate autosampler for chiral HPLC-MS analysis.

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