Available online at www.sciencedirect.com
SCIENCE DIRECT®
Microchemical Journal 76 (2004) 105–112
MICROCHEMICAL JOURNAL
www.elsevier.com/locate/microc

Evaluation of laser-based polarimetry for the determination of enantiomeric excess (ee) at the extremes of the ee scale

Sean W. Linder[a], Gary W. Yanik[b,1], Donald R. Bobbitt[a,*]

[a]*Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701, USA*
[b]*PDR-Chiral, Inc., 1331A South Killian Drive, Lake Park, FL 33403, USA*

Received 23 September 2003; accepted 10 October 2003

Abstract

The development of new approaches for assessing enantiomeric purity is an area of critical need in the pharmaceutical and biotechnological industries. Recent work has shown that two enantiomers, although virtually identical physically, may exhibit widely divergent physiological properties. In extreme situations, the contaminant enantiomer may itself be physiologically active leading to significant health consequences. Laser-based polarimetric detection, in combination with a chiral-selective separation mode, has been shown to be capable of assessing enantiomeric purity in situations where the two enantiomers exist as either a racemic or near racemic mixture. The laser-based polarimetric detector responds directly to the intrinsic optical activity of the eluting analyte. The sign of the response can provide unique information with respect to which enantiomer, of the enantiomeric pair is eluting. The detector has been shown to be sensitive and linear under analytical conditions. However, this combined methodology has not been rigorously evaluated under conditions of low enantiomeric excess (ee). Such situations would be encountered, for example, when one is attempting to characterize a pharmaceutical preparation marketed as enantiomerically pure. In this work, laser-based polarimetric detection, in combination with a chiral-selective separation mode will be evaluated under conditions at the extreme of the ee scale. Under such conditions, this approach will be shown to be capable of making measurements below 0.2%ee, even in the presence of a 1000-fold excess of the major enantiomer. UV/Vis detection, evaluated under identical conditions, could not accurately predict the ee even when baseline resolution of the enantiomers was possible. This was attributed to the presence of absorbing impurities that can co-elute with the minor enantiomer. Since the polarimetric detection system responds only to optically active analytes, these impurities do not affect the quality of the enantiomeric measurement.

Keywords: Enantiomeric excess (ee); Laser-based polarimetry; Enantiomers

1. Introduction

Enantiomers are defined as non-superimposable mirror image molecules and provide a number of targets for pharmaceutical and biotechnology research and development. Enantiomers often exhibit different biological activity in living organisms, thus driving the need for analytical methodology appropriate for the rapid and precise determination of enantiomeric purity. For example, thalidomide was marketed as a racemate in the 1960s for the suppression of morning sickness and when distributed, caused severe birth defects in newborn children. The non-therapeutic enantiomer was later found to be metabolized into a teratogenic compound in humans. Thus the separation of enantiomers and/or the development of enantio-selective synthetic methods for production must be carefully monitored in order to assure safety.

Currently, commercially manufactured chiral molecules are synthesized via either symmetric or asymmetric synthesis. The most time efficient trend is to develop a symmetric synthetic protocol for the production of both enantiomers. Once this has been accomplished and a

*Corresponding author. Tel.: +1-501-575-4804; fax: +1-501-575-2642.
E-mail address: dbobbitt@uark.edu (D.R. Bobbitt).
[1] PDR-Chiral, Inc., 1331A South Killian Drive, Lake Park, FL 33403, USA

0026-265X/04/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.microc.2003.10.006

patent application has been submitted, research into an asymmetric synthetic approach often begins. This method of developing a symmetric process followed by asymmetric synthesis is commonly termed the racemic switch. It is advantageous for pharmaceutical or biotechnology firms to implement such methods in order to prevent competitors from developing synthetic approaches for either the pure enantiomers or racemates. Such competition has been discussed in detail in a recent article in Chemical and Engineering News [1,2].

Unless a chiral molecule is marketed as a racemate, new technologies and methodologies are needed for enantiomeric analysis in order to meet new criteria for purity assessment. In the case of symmetric synthesis, large-scale enantio-selective separation systems, such as simulated moving bed [3–6], super-critical fluid [7], and prep-scale [8–10] high-performance liquid chromatography must be implemented in order to produce the pure enantiomers in large quantities with a high level of selectivity. However, once the large-scale separation is complete, an analytical separation and detection method must be implemented in order to quantitate, with high accuracy and precision, the enantiomeric content. A similar enantiomeric purity determination is required when using asymmetric synthesis, however, with asymmetric synthesis, large-scale separation of enantiomers is not required.

This accurate and precise determination of enantiomeric content has become necessary, not only due to the difference in bioactivity of each enantiomer, but also because the Food and Drug Administration has implemented strict guidelines with respect to the identification and quantitation of chiral pharmaceutical candidates. The sale of single-enantiomer drugs has steadily increased over the last decade and it is estimated that by 2005, sales will reach 171 billion dollars or 44% of total drug sales [2]. With the rapid increase in single-enantiomer drugs, the pharmaceutical industry is looking for new alternatives for the analysis of enantiomeric content, especially when typical methods of analysis do not provide adequate sensitivity, such as for a drug candidate that does not contain an ultraviolet absorbing chromophore or fluorophore.

The primary indicator of enantiomeric content is the determination of enantiomeric excess (ee). Enantiomeric excess is defined by the following equation:

$$\%\text{ee} = \frac{m_+ - m_-}{m_+ + m_-} \times 100 \quad (1)$$

where m_+, represents the mass of the (+) enantiomer and m_-, represents the mass of the (−) enantiomer. Thus, the closer the value is to 100%, the greater the enantiomeric purity. This calculation provides a numerical assessment of the enantiomeric content based on the relative masses of each individual enantiomer. Based on Eq. (1), the sign of the %ee determines which of the two enantiomers exists in excess.

The assessment of enantiomeric excess can be accomplished using either an enantio-selective separation procedure followed by detection [11–13], or simultaneous determination of the ratio of the two enantiomers using direct, enantio-selective detection [14]. Currently, the most common method employed for the determination of enantiomeric excess utilizes a chiral chromatographic separation procedure. These methods can be used with several different commercially available detectors and the only requirement for quantitation is the presence of baseline enantiomeric resolution. The most commonly used detection method is UV/Vis absorbance detection due to its versatility for a wide range of analytes, however, it requires that an absorbing chromophore be present in the target analyte. In addition to UV/Vis absorbance detection, circular dichroism (CD) detection, which responds directly to the chirality of a given molecule, has also been used for detection of enantiomers following chiral selective separation [15,16]. CD offers the advantage of chiral selectivity, however, it also requires that the absorbing chromophore and the chiral center be within close electronic proximity for efficient detection. With its implementation as a commercially available detector for HPLC, fluorescence has created a niche due the increased sensitivity of approximately one order-of-magnitude over other absorbance detection methods [17]. However, the addition of a fluorophore either pre- or post-column can be time consuming, expensive and problematic. Thus either an increase in instrument sensitivity or selectivity must be justified in order for implementation of HPLC compatible fluorescence detection. With the recent commercialization of a turn-key detector, laser-based polarimetry has also become a popular detector for enantiomeric analysis due to its chiral-selective response [11,18]. The only requirement for laser-based polarimetric detection is the presence of optical activity. However, it is often the case that laser-based polarimetry is less sensitive than UV/Vis and fluorescence absorbance detection.

Prior to the development of chiral selective separation methods, the use of achiral methodologies utilizing both laser-based polarimetry and circular dichroism for the assessment of enantiomer excess were the primary methods of analysis [14,19]. The general disadvantage of most achiral methods are the relatively poor detection limits with the majority of achiral methods only quantitating at, or above the 2%ee level. Two relatively new detection methods for achiral determination of ee are mass spectrometry (MS) [20] and nuclear magnetic resonance spectroscopy (NMR) [21]. They both offer ee quantitation in the 2–4% range. MS has an increased potential to become a component of chiral detection methodologies when coupled with HPLC, however, the chief concern is that the HPLC eluent has the potential

to interfere with the MS determination, especially when utilizing electrospray ionization.

The focus of this work will be the assessment of enantiomeric excess with chiral selective HPLC using both ultraviolet absorbance and laser-based polarimetric detection under conditions of extreme ee (>99%) values. UV/Vis absorbance detection derives its response from Beer's Law as follows:

$$A = \varepsilon bc \tag{2}$$

where ε (M^{-1} cm^{-1}) is the molar absorptivity, which is constant for a given wavelength, b is the path length (cm), and c (M) is the concentration of the analyte. The response for laser-based polarimetry can be described by a similar equation, as follows:

$$\alpha = [\alpha]bc \tag{3}$$

where α (deg) is the observed rotation, $[\alpha]$ is the specific rotation (deg dm^{-1} $(g/ml)^{-1}$), which is a constant for a given wavelength, temperature, and solvent, b is the path length (dm), and c is the concentration (g/ml). When considering an equal path length and concentration for the two detectors, the only variables are the molar absorptivity and specific rotation. Thus, it is conceivable that the magnitude of these two constants will be directly proportional to the instruments sensitivity, with respect to a given analyte.

In order to accurately quantitate extreme ee (>99%) values using HPLC coupled with UV/Vis absorbance and laser-based polarimetric detection, several chromatographic parameters become vitally important for success. The two main parameters that must be optimized for ee determination are enantiomeric resolution (r_s) and capacity factor (k'). Enantiomeric resolutions greater than 1.0 is required for accurate integration of each enantiomeric chromatographic peak or distribution, thus eliminating the possibility that peak overlap could interfere with quantitation. The capacity factor represents another important chromatographic parameter for extreme %ee assessments, due to the probability that impurities or anomalous responses could be present at the dead volume of the chromatographic column; thus a k' value of 1.0 or greater would be ideal. Once the chiral chromatographic parameters have been optimized, the detection methods can be evaluated under such conditions.

Both UV/Vis and fluorescence spectroscopic methods have the potential for quantitation at, or below the 1%ee level, however, both are mass dependent detection methods and, in the case of absorbing impurities or non-baseline resolution, precise peak integration can become problematic. When considering the close proximity of retention times characteristic of chiral chromatography for enantiomeric pairs, coupled with the fact that 99%ee equates to a relative ratio of 200:1 for the two enantiomers, the possibility of absorbing interferences from impurities or non-baseline chromatographic resolution is greatly increased. Thus, the utilization of a detector that only responds to optically active materials could potentially allow the quantitation of peak area in the presence of non-optically active impurities, as well as quantitation under conditions with non-baseline enantiomeric resolution. To date, no chiral selective detection method has demonstrated the ability to quantitate with high precision at the <2%ee level. In this work, laser-based polarimetric detection will be shown to be capable of detecting and quantitating at an ee of 0.2%, or below. Based on the signal-to-noise ratio (S/N) of the detector during these experiments, it is conceivable that a level of 0.1%ee could be detectable. Furthermore, for the chosen probe, mandelic acid, polarimetry will be shown to be a more sensitive detection method when compared to UV/Vis absorbance detection.

2. Experimental

All chromatographic experiments were performed using a Hewlett–Packard high-performance liquid chromatography (Model 1100, Palo Alto, CA) system coupled in-series with a PDR-Chiral, Inc. laser-based polarimeter (Palm Beach Gardens, FL). Detection cell volumes for the absorbance and polarimetric detectors were 14 μl and 21.5 μl, respectively. The effluent from the separation column was directed first through the polarimeter ($\lambda_{detection}$ = 670 nm), and then, in-series to the UV absorption detector ($\lambda_{detection}$ = 254 nm). The UV absorbance was measured at 254 nm for all experiments. The mobile phase for all experiments was composed of 1% triethylammonium acetate (TEAA) buffer at pH 4.0, at a flow rate of 1.00 ml/min. A Chirobiotic T (Advanced Separation Technologies Inc., Whippany, NJ) chiral chromatography column (250×4.6 mm) was used for chiral separation of the enantiomers.

Mandelic acid was purchased from Aldrich Chemical Company (Milwaukee, WI) in 99+% enantiomerically pure form. The mandelic acid was dissolved in the 1% TEAA buffer at pH 4.0 in order to create standard solutions for both of the pure enantiomers. Varying concentrations and enantiomeric excesses were prepared by volumetric dilution from the concentrated standards.

Raw data were collected on a PC at a rate of 2 Hz after digitization using an external Hewlett–Packard 35900 E A/D converter. All data processing was performed using Microsoft Excel and HP Chemstation software.

3. Results and discussion

With the growing need for rapid high precision measurement of enantiomeric excess, new analytical

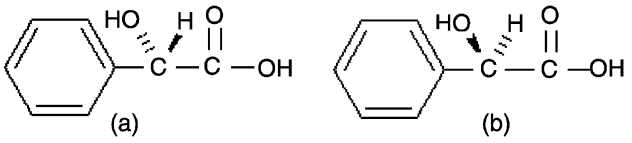

Fig. 1. Molecular structure of (S)-(+)-mandelic acid (a) and (R)-(−)-mandelic acid (b)

Table 1
Effects of mobile phase composition on enantiomeric resolution

eOH (%)	1% TEAA (%)	R_s	α	k'
30	70	9.14	3.64	0.38
20	80	7.64	2.98	0.52
10	90	7.15	2.59	0.64
0	100	6.26	2.18	0.87

instrumentation and methodologies must be developed to address this challenge. Ultraviolet spectroscopy has been the most commonly used approach for ee determination; however, not all chiral analytes contain UV active chromophores. Thus, the use of other, more selective optical detectors such as laser-based polarimetry as an adjunct to UV spectroscopy has become of significant interest to the scientific community, especially when considering the potential for improved sensitivity for optically active analytes with low molar absorptivity values [22–24].

The model analyte chosen for this study was mandelic acid due to its moderate optical activity and UV/Vis absorbance. The molecular structure of the enantiomers of mandelic acid are depicted in Fig. 1. Mandelic acid standards were readily available in 99+% purity for both enantiomers and an established chiral chromatographic separation method was available from the chromatographic column manufacturer, Advanced Separation Technologies Inc. (ASTEC). This separation methodology would serve as a starting point for the optimization of the chromatographic retention parameters that drive the success of high precision enantiomeric excess determinations.

Calibration data were experimentally obtained for mandelic acid, using both the ultraviolet absorbance and laser-based polarimetric detectors, as shown in Fig. 2. This calibration data provides assurance that both detectors provide a linear response over the injected mass range of interest. It was experimentally determined that both detectors provided a linear response, $R^2 = 0.999$, from 1.0 to 120 μg of mandelic acid under the optimized chromatographic conditions. The calibration data were collected in triplicate for each injection mass to ensure that adequate precision (<3% RSD) and reproducibility were obtained.

Once the calibration data set was obtained, studies into the effect of mobile phase composition on the chromatographic enantiomeric resolution (R_s), relative retention (α), and capacity factor (k') were performed with racemic mandelic acid. The results are summarized in Table 1. From this data, one can conclude that a decrease in the percentage of methanol in the mobile phase subsequently decreases the enantiomeric resolution and relative retention. However, the opposite effect occurs with respect to the capacity factor; a decrease in methanol provides an increase in capacity factor. It was important to choose a proper balance of mobile phase composition that both provided substantial enantiomeric resolution and a reasonable (>1.0) capacity factor. Thus, a 80% TEAA buffer and 20% methanol composition was chosen for the high precision experiments due to consideration of both retention characteristics and enantiomeric resolution.

With the acquisition of calibration data and optimization of mobile phase composition, chromatographic experiments were designed in order to maximize the accuracy and precision for the determination of %ee. It was found that in order to provide the most accurate samples for analysis, large volumes of standard solutions of each enantiomer must be prepared in order to limit inaccuracies obtained via balance transfer and mass

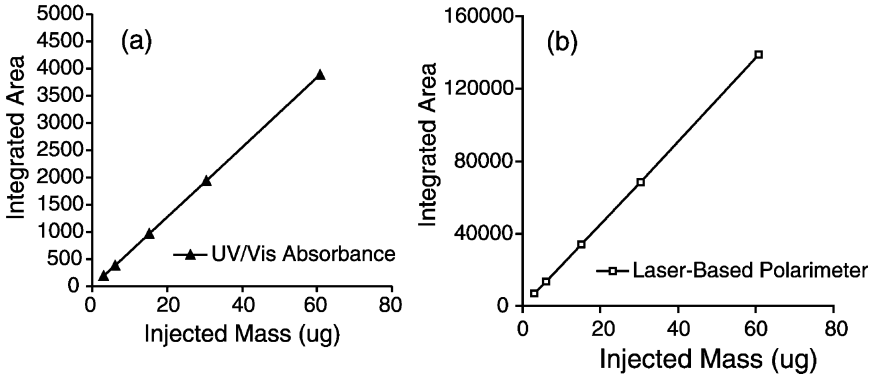

Fig. 2. Linear calibration plots for (R)-(−)-mandelic acid utilizing UV/Vis absorbance detection (a) and laser-based polarimetric detection (b).

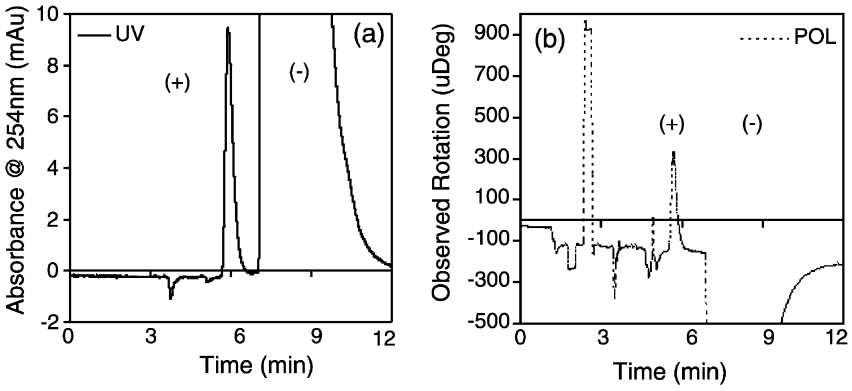

Fig. 3. UV/Vis (a) and laser-based polarimetric (b) detector responses for a chromatographic injection of 20 μl of a 98.00%ee mandelic acid standard. The mandelic acid enantiomers were separated on a Chirobiotic T 250×4.6 mm chromatography column at a flow rate of 1.00 ml/min with a mobile phase composition of 1% TEAA pH 4.0.

measurements. The first eluting enantiomer, (+)-mandelic acid, would be designated the minor impurity with the (−)-mandelic acid receiving the designation of the major enantiomer of interest. With these designations, stock solutions were prepared at a predetermined concentration so that mixing the solution together at equal volumes would provide the desired enantiomeric excess. Once these stock solutions were mixed together, subsequent quantitative analysis via HPLC with UV/Vis absorbance and laser-based polarimetric detection could be evaluated at the given enantiomeric excess level. It is important to note that the minor enantiomer must be prepared in large volumes due to the relatively dilute concentrations required for extreme enantiomeric excess.

The initial set of experiments focused on the ability of laser-based polarimetry to quantitate enantiomeric excess at a level of 98.0%, or 1 part of the minor enantiomer per 100 parts of the major enantiomer. It was determined that evaluating this methodology on a more macro %ee scale would allow the determination of its viability as a detection approach. Thus, stock solutions of 2 mM (+)-mandelic acid and 200 mM (−)-mandelic acid were prepared by dissolving the pure standards in the 1% TEAA buffer at pH 4.0. Once prepared, the stock solutions were mixed at equal volumes using volumetric pipettes in order to maximize the accuracy and precision, and then subsequently analyzed via HPLC.

Fig. 3a represents the UV/Vis absorbance response signal obtained via a single chromatographic analysis of 98.0%ee mandelic acid separated on a Chirobiotic T chiral chromatography column at a flow rate of 1.00 ml/min and a mobile phase consisting of 1% TEAA buffer at pH 4.0. This figure is scaled in order to emphasize the signal corresponding to the minor eluting (+)-mandelic acid enantiomer ($t_R \approx 6.0$ min.). Fig. 3b represents the laser-based polarimetric response signal obtained for the same injection, with the minor enantiomer eluting at approximately 5.5 min. It is important to note the DC calibration signals of −100 and +1000 μ deg obtained via the internal standard for the laser-based polarimeter at a retention time of approximately 2–3 min. This calibration signal based on the Faraday effect allows the user to confirm the accuracy of the polarimeter's response signal in real time and track changes for normalization. In both Fig. 3a,b, the minor enantiomer exhibits peak area sufficient for integration with baseline resolution and adequate capacity factor. Under these experimental conditions, quantitation of the minor enantiomer should provide little challenge for either detector.

Using the previously obtained regression data for mandelic acid, the %ee was experimentally calculated from the integrated peak areas for a series of 10 consecutive chromatograms obtained via injections of the prepared 98.00%ee samples. It is possible to determine the mass associated with the minor eluting enantiomer and subsequently obtain a %ee calculation. Upon integration of peak areas and subsequent calculation of %ee for the 10 chromatograms, it was determined that the experimental %ee was equal to 98.00±0.01% and 98.16±0.04% for the UV/Vis absorbance and laser-based polarimeter, respectively. From these calculations, it is apparent that the accuracy and precision of both the UV/Vis absorbance and laser-based polarimetric detectors are extremely high. Chromatogram reproducibility and peak area integration facilitated this high accuracy and precision. With the success at the 98.00%ee level, solutions were prepared in order to create a 99.8%ee sample for further evaluation of this methodology.

Fig. 4a represents the response obtained from the UV/Vis absorbance detector from a 20 μl injection of a 1:1 mixture of 0.2 mM (+)-mandelic acid and 200.0 mM (−)-mandelic acid separated on a Chirobiotic T chiral chromatography column at a flow rate of 1.00

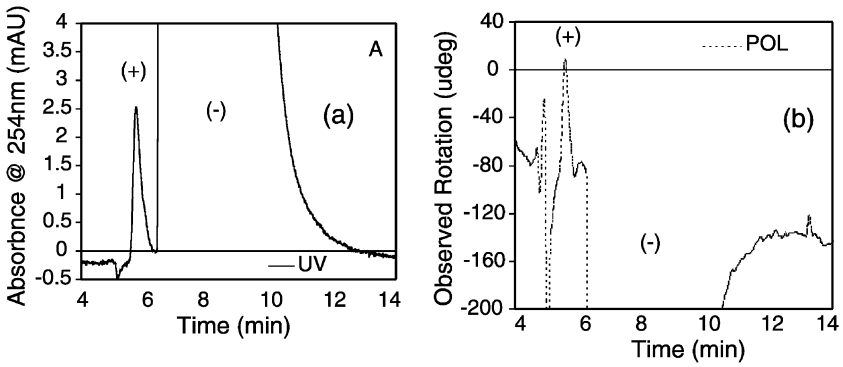

Fig. 4. UV/Vis (a) and laser-based polarimetric (b) detector responses for a chromatographic injection of 20 μl of a 99.80%ee mandelic acid standard. The mandelic acid enantiomers were separated on a Chirobiotic T 250×4.6 mm chromatography column at a flow rate of 1.00 ml/min with a mobile phase composition of 1% TEAA pH 4.0.

ml/min and a mobile phase consisting of 1% TEAA buffer at pH 4.0. Using this mixture of enantiomers, the theoretical %ee was calculated to be 99.80% or one part of (+)-mandelic acid per 1000 parts of (−)-mandelic acid, assuming that both the mandelic acid standards were 100% enantiomerically pure. The first eluting chromatographic peak corresponds to the (+)-mandelic acid, followed by the (−)-mandelic acid. As seen previously, the (+)-mandelic acid could be considered the minor enantiomeric impurity due to its relatively small injected mass when compared to the (−)-mandelic acid. With the absorbance detector, integration of the peak areas is possible for the quantitation of %ee due to the chromatographic resolution of approximately 1.0 for the two enantiomeric distributions. Upon integration of peak area and subsequent calculation of %ee for the ten chromatograms, it was determined that the experimental %ee was equal to 99.73±0.01%. Due to the large chromatographic tailing effect from the major eluting enantiomer as seen in Fig. 4, it becomes apparent, that in order maximize peak integration of the minor enantiomer, the experiment should be designed so that the minor enantiomer elutes first.

Fig. 4b represents the response obtained from the laser-based polarimetric detector from the same sample of 99.80%ee mandelic acid. The chromatographic resolution is such that integration of the minor enantiomer is possible, unfortunately integration of the major enantiomeric peak cannot be accomplished because the dynamic range of the instrument was exceeded causing clipping of the top of the peak distribution. Thus, a relative ratio of the two peak area distributions cannot be used to determine the relative mass ratios and subsequent %ee calculations. Therefore, one must integrate only the area that corresponds to the minor eluting enantiomer and convert this value to mass. Once the mass of the minor eluting enantiomer has been determined, subsequent %ee calculations can precede based on the total mass injected of mandelic acid. The calculated %ee was found to be 99.73±0.01% when using laser-based polarimetric detection. This experimentally determined value for enantiomeric excess agreed very well when compared to the ultraviolet absorbance detector and the theoretical evaluation of the contrived sample.

For the previous calculations of enantiomeric excess, it was assumed that the mandelic acid standards were 100% enantiomerically pure due to the fact that the supplier did not perform quantitation of the standards at levels greater than 99%. In order to compare the %ee obtained from the absorbance and laser-based polarimetric detectors and the theoretical %ee, the absolute enantiomeric purity for the mandelic acid standards must be evaluated. This was accomplished using the same chiral separation system previously utilized, however, injections of the standards were made independent of each other in order to gain an absolute purity assessment for each. With the previously obtained calibration data, quantitation of the two eluting peaks if present, would provide a measure of the absolute purity of the standards. The major focus of this purity assessment was the (−)-mandelic acid because it was considered the major eluting enantiomer in all of our studies and represented the greatest percentage of injected mass. Any impurities in the major eluting enantiomer, whether chiral or achiral, could potentially contribute to the mass associated with the minor eluting enantiomer and subsequently lower both the accuracy and precision of the obtained %ee values. It was found from the ultraviolet absorbance detector that the (−)-mandelic acid had an enantiomeric purity of 99.97% (wt/wt) based on the previously obtained calibration plot. The peak area associated with the impurity in (−)-mandelic acid exhibited the same retention time as (+)-mandelic acid. Thus, for every injection of (−)-mandelic acid, 0.03% was assumed to be (+)-mandelic acid based on the chromatographic evidence.

Once the contribution of the enantiomeric impurity from the (−)-mandelic acid was considered, the experimentally determined %ee, based on Fig. 4, was calculated to be 99.77 ±0.01% and 99.80±0.02% for the UV/Vis absorbance and laser-based polarimeter, respectively. Thus, when comparing the experimentally determined %ee from both the absorbance and laser-based polarimetric detectors to the theoretical %ee, the detectors provided accurate and precise %ee assessments when used in conjunction with a chiral separation system. This accuracy and precision can be attributed to the high degree of linearity and high signal-to-noise ratio for both detectors over a large range of injected masses.

The use of either an absorbance or laser-based polarimetric detector for the rapid screening of closely related enantiomeric excess values can be a valuable analytical tool for the pharmaceutical and biotechnology industries. Rapid screening could be of major importance when considering if an enantiomerically purified product meets quality control and assurance specifications or requires further purification processing. The difference between 99.6%ee and 99.8%ee could potentially equate to the difference between having a marketable product, or having one that requires further purity processing. Fig. 5A illustrates the minor enantiomer, (+)-mandelic acid, of two subsequent injections of 99.6% and 99.8%ee mandelic acid using an ultraviolet absorbance detector. It is apparent that the magnitude of the two responses are very similar, thus implying that rapid assessment of enantiomeric excess would not be possible using this detector even if a chiral separation existed and calibration data was previously obtained. The peak height or peak area for the absorbance signal from the 99.8%ee sample does not have the one-half relationship with the 99.6%ee sample signal one would expect. This can be attributed to either the small response slope generated for mandelic acid using the ultraviolet absorbance detector at 254 nm under these specific chromatographic conditions, or it may possibly indicate contributions from non-optically active materials that co-elute with the analyte.

Fig. 5b shows the same injections of 99.6 and 99.8%ee using a laser-based polarimetric detector. The response generated from the minor enantiomer appears to be approximately one-half in peak height and peak area. This can be attributed to a much larger response slope for mandelic acid under these specific chromatographic parameters. With its large slope, the laser-based polarimeter would appear to be a more sensitive and selective detector, when compared to the ultraviolet absorbance detector at 254 nm, for mandelic acid under the conditions listed previously. With its large response slope and adequate signal-to-noise ratio, the laser-based polarimeter appears to be a powerful analytical tool for the rapid determination of closely related %ee values under the given chromatographic conditions.

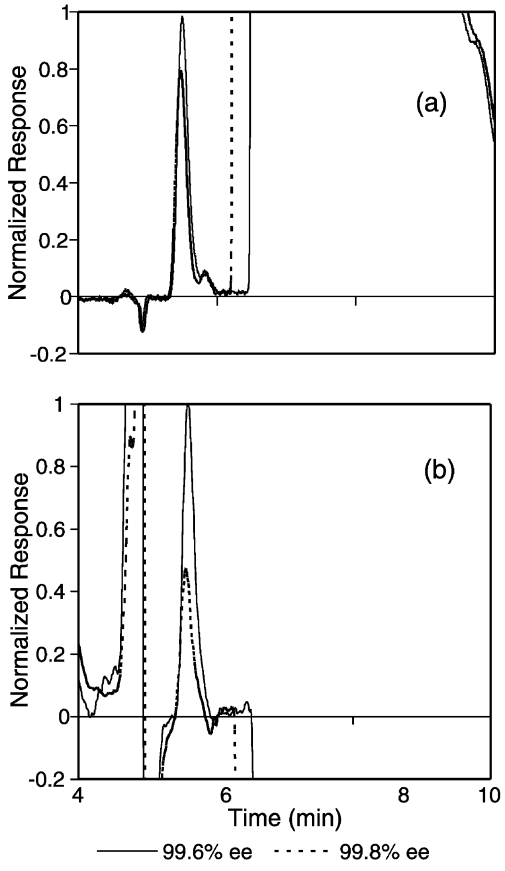

Fig. 5. Comparison of normalized response obtained from 99.6 and 99.8%ee mandelic acid injections using UV/Vis (a) and laser-based polarimetric and (b) detector responses.

The use of laser-based polarimetry has been proven to provide accurate and precise quantitative information with respect to extreme enantiomeric excess determinations when chiral separation has occurred. This technique could provide a powerful adjunct to an absorbance detector when used in high-performance liquid chromatographic separation systems for the analysis of analytes with small molar absorptivities. The use of mandelic acid for these experiments is an ideal case for laser-based polarimetry, however, analytes with significantly smaller specific rotations should follow the methodology provided the chiral separation conditions meet the requirements previously stated. Under these circumstances, significantly larger injection masses could be used in place of a large specific rotation. This methodology could also be useful for compounds that have relatively small molar absorption constants, but large specific rotation values.

It must be reiterated that detection at the one part per thousand level for a minor enantiomer in the presence of a major enantiomer is a significant analytical achieve-

ment, especially when considering the fact that enantiomers are virtually identical compounds. The only structural difference in the two enantiomers of mandelic acid is a subtle change in the molecular configuration at one carbon (out of eight) located directly adjacent to the aromatic ring. Thus, enantiomeric separation is a difficult task itself, however, the ability to separate and detect enantiomeric compounds at a relative enantiomeric ratio of 1:1000 is a challenging measurement, but one that potentially could have very significant application in both the pharmaceutical and biotechnology industries. The use of a chiral-selective detection method, i.e. laser-based polarimetry, for such extreme %ee determinations has an inherent advantage due to its ability to respond directly to optically active materials. In comparison, absorbance detection methods respond directly to all absorbing materials, chiral or achiral, present in the detection cell for a given probing wavelength. This is an important difference due to the fact that pharmaceutical formulations often contain a variety of trace impurities and major component incipients; however, these impurities are generally achiral. Laser-based polarimetric detection thus has been shown to be advantageous for the enantiomeric quantitation at the extreme ranges of the %ee scale, and could potentially serve as the basis for purity certification of pharmaceutical formulations.

Acknowledgments

The authors acknowledge the Center for Process Analytical Chemistry, University of Washington for financial support of this work.

References

[1] S.C. Stinson, Chem. Eng. News 78 (2000) 55–78.
[2] S.C. Stinson, Chem. Eng. News 79 (2001) 79–97.
[3] M. Juza, J. Chromatogr. A 865 (1999) 35–49.
[4] L.S. Pais, J.M. Loureiro, A.E. Rodrigues, Sep. Purif. Technol. 20 (2000) 67–77.
[5] J. Haag, A.V. Wouwer, S. Lehoucq, P. Saucez, Control Eng. Practice 9 (2001) 921–928.
[6] M. Schulte, J. Strube, J. Chromatogr. A 906 (2001) 399–416.
[7] K.W. Phinney, Anal. Chem. (2000) 204A–211A.
[8] F.W Collins, J. Clin. Ligand Assay 23 (2000) 273–282.
[9] F. Geiser, M. Schultz, L. Betz, M. Shaimi, J Lee, W. Champion Jr., J. Chromatogr. A 865 (1999) 227–233.
[10] C.I. Keeling, H.T. Ngo, K.D. Benusic, K.N. Slessor, J. Chem. Ecol. 27 (2001) 487–497.
[11] R.G. Lodevico, D.R. Bobbitt, T.J. Edkins, Talanta 44 (1997) 1353–1363.
[12] N.R. Srinivas, L.N. Igwemezie, Biomed. Chromatogr. 6 (1992) 163–167.
[13] E.R. Francotte, J. Chromatogr. A 906 (2001) 379–397.
[14] K. Kudo, K. Iwata, C. Yomota, S. Morris, M. Saito, Enantiomer 5 (2000) 369–375.
[15] F. Zsila, A. Gergely, P. Horvath, G. Szasz, J. Liq. Chromatogr. Relat. Technol. 22 (1999) 713–719.
[16] C. Bertucci, V. Andrisano, V. Cavrini, E. Castiglioni, Chirality 12 (2000) 84–92.
[17] D.C. Harris, Quantitative Chemical Analysis, 5th ed, W.H. Freeman and Company, New York, 1999.
[18] Y.-S. Liu, T. Yu, D.W. Armstrong, Lc-Gc 17 (1999) 946, 948, 950, 952, 954, 956–957.
[19] A. Gergely, Prog. Pharm. Biomed. Anal. 4 (2000) 553–561.
[20] W.A. Tao, F.C. Gozzo, R.G. Cooks, Anal. Chem. 73 (2001) 1692–1698.
[21] R. Rothchild, Enantiomer 5 (2000) 457–471.
[22] K. Ng, P.D. Rice, D.R. Bobbitt, Microchem. J. 44 (1991) 25–33.
[23] S.W. Linder, G.W. Yanik, E.R. Francotte, D.R. Bobbitt, Enantiomer 7 (2002) 41–47.
[24] K. Ng, S.W Linder, J. Chromatogr Sci. 41 (2003) 460–466.