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Circular dichroism detection in high-performance liquid chromatography: evaluation of the anisotropy factor*

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Abstract: The application of a circular dichroism (c.d.) detection system in HPLC using a chiral stationary phase is presented. The simultaneous measurement of the absorbance and c.d. signal allows the evaluation of the anisotropy factor ($g = \Delta\epsilon/\epsilon$) and thus the determination of the enantiomeric excess (e.e.) of the eluates. When this detection system is used in preparative chiral chromatography the collection of the enantiomeric fractions can be readily optimized.

Keywords: *Circular dichroism; liquid chromatography detection system; chiral discrimination; drug analysis; stereochemistry; Pirkle phase; g-factor; enantiomeric excess; preparative LC.*

Introduction

In the last few years the growth of chiral chromatography has made a major impact on stereochemical analysis [1–6]. In this context the obvious detection system is that based on chiroptical properties. Indeed, several papers have appeared in the literature concerning either the measurement of optical rotation [7, 8] or circular dichroism (c.d.) [8–13]. This paper describes a c.d. detection system, obtained by modifying a commercially available c.d. spectropolarimeter, which enables the absorbance and c.d. signal to be monitored simultaneously. This technique, first introduced by S.F. Mason *et al.* [9], allows the anisotropy factor ($g = \Delta\epsilon/\epsilon$) to be evaluated, thus enabling the enantiomeric excess (e.e.) to be calculated. The direct measurement of the e.e. gives fundamental data to facilitate the optimization of fraction collection in preparative liquid chromatography.

Experimental

Chemicals

(*R*,*S*)-7-chloro-1,3-dihydro-3-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one, (*R*,*S*)-**1**, was kindly provided by Professor W.H. Pirkle (School of Chemical Sciences, University of Illinois at Urbana-Champaign, USA).

Chiral stationary phases

The chiral stationary phase "SiSquinmei" (CSP I) was obtained by reacting N-methylquininium iodide with γ-mercaptopropylsilanized silica, prepared starting from LiChrosorb Si60 (5 μm, Merck, Darmstad, FRG) and (3-mercaptopropyl)-trimethoxysilane, as described elsewhere [14]. The chiral stationary phase "SiSquindmei" (CSP II) was obtained following the same procedure adopted for CSP I, but using *N*-methylquinidinium iodide as chiral derivatizing agent [15]. Two 125 × 4.6 mm i.d. columns were slurry packed (MeOH) with the CSPs by conventional techniques. The Pirkle column [i.e. (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine ionically bonded to a commercial 5-μm γ-aminopropylsilanized silica column from Merck (Darmstadt, FRG)], CSP III, was prepared *in situ*, following the procedure reported previously [16].

Chromatographic resolution

The separations were carried out using a Jasco Twincle apparatus. Simultaneous UV and c.d. detection was carried out using a Jasco J-600 spectropolarimeter (set at 254 nm),

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equipped with a micro HPLC cell (volume 8 μl) and a doublet of lenses to focus the light beam in the sample compartment. The evaluation of the anisotropy factor was obtained by personal computer processing of the absorbance and c.d. data. All the solvents used for the preparation of the mobile phase were HPLC grade and the mixtures were filtered and degassed before use.

Results and Discussion

Chiral chromatography can give considerable assistance when pharmacological tests have to be carried out on the enantiomers of a chiral drug available as a racemic mixture. In fact, fractions of enantiomers for pharmacological studies are readily obtained if preparative chromatographic resolution is performed [17–20]. In this respect c.d. detection is of critical importance, since it allows direct evaluation of the actual enantiomeric composition, in addition to the direct determination of the absolute configuration of the eluates [11]. Thus it is possible to optimize fraction collection even when the two enantiomer peaks partially overlap. The enantiomeric excess (e.e.) can be determined by simultaneously monitoring the absorbance and the dichroic signal and by evaluating their ratio (anisotropy factor, $g = \Delta\epsilon/\epsilon$) [9]. When the value of the *g*-factor of the pure enantiomer is already known, the experimental value is directly related to the e.e. (e.e. = $g_{exp}/g_{max} \cdot 100$). An example of the use of this detection system is shown in Fig. 1, where the chromatographic resolution of **1** on CSP III is reported. In this case, the value of the anisotropy factor is constant and reversed in sign for the two enantiomers.

When baseline resolution is obtained, as in the case of Fig. 1 (e.e. is 100% for both the enantiomers), the point at which the fractions should be collected is obvious. The *g*-factor value obtained represents the g_{max} of the enantiomers of **1** at 254 nm, under the experimental conditions adopted. However, preparative chromatography usually presents the problem of partial overlap of the two peaks, because of the decrease in efficiency due to the increased sample loading and/or column dimensions. When partial resolution is obtained, as reported in Fig. 2, the e.e. of the eluted fractions can be determined by the profile of the *g*-factor, since e.e. and *g* are linearly related. It can be readily seen that the first eluted enantiomer can be collected at high optical purity, while the second cannot. Nevertheless, for both enantiomers the value of the *g*-factor permits one to establish the largest volume of the fractions that should be collected without significantly changing their e.e. In this case (Fig. 2) the first eluted enantiomer has a value of e.e. ≥95% between 400–420 s, whilst the more strongly retained enantiomer can be collected between 440–480 s with an e.e. of around 60%.

Of course, as regards the analytical deter-

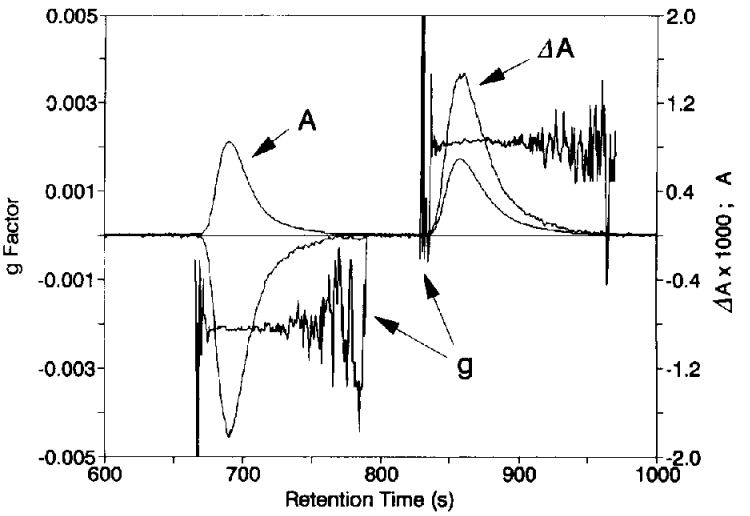

Figure 1
Chromatographic resolution of **1** on CSP III. Eluent hexane–2-propanol (90:10, v/v); flow rate 1 ml min^{-1}. UV, c.d. and *g* detection at 254 nm.

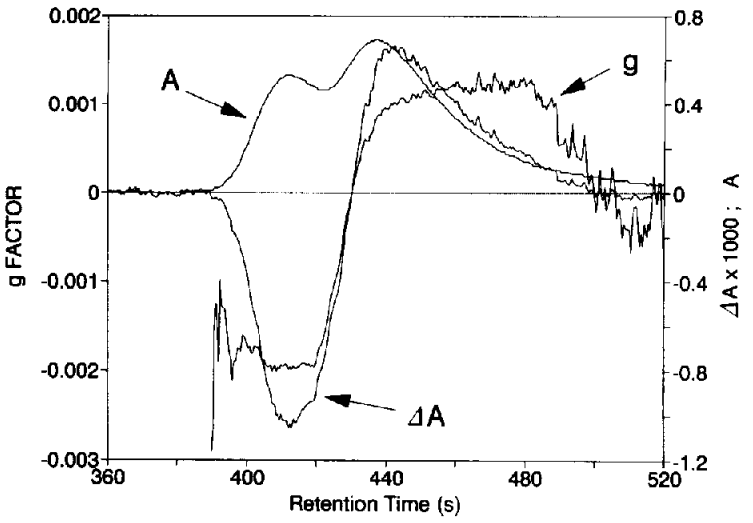

Figure 2
Chromatographic resolution of **1** on CSP I. Eluent hexane–2-propanol–CH_2Cl_2 (88:2:10, v/v/v); flow rate 1 ml min^{-1}. UV, c.d. and *g* detection at 254 nm.

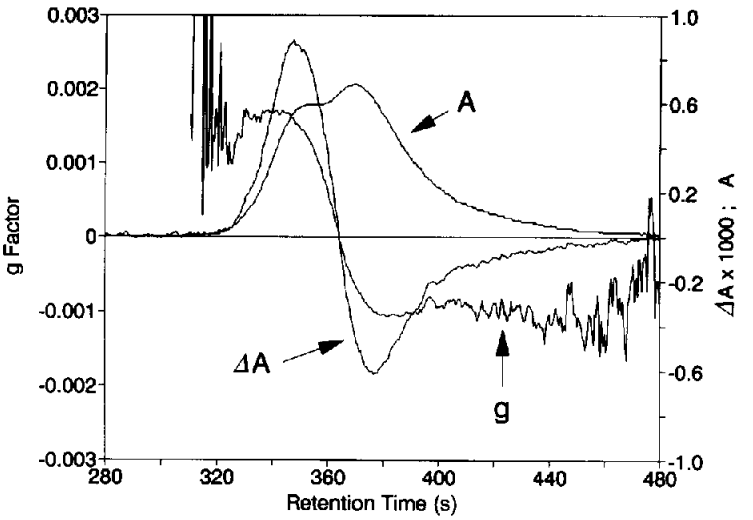

Figure 3
Chromatographic resolution of **1** on CSP II. Eluent hexane–2-propanol–CH_2Cl_2 (88:2:10, v/v/v); flow rate 1 ml min^{-1}. UV, c.d. and *g* detection at 254 nm.

mination of enantiomeric composition, the availability of chiral stationary phases with reversed chirality and therefore elution order is remarkably helpful. Indeed, this allows the two enantiomers to be obtained in practically pure form, by arranging to collect the first eluted enantiomer on each of the two columns. In Fig. 3 the chromatographic resolution of compound **1** on CSP II is reported. CSP II is quasi-enantiomeric with respect to CSP I so that, indeed, the elution order is reversed.

The c.d. detection system reported here presents the particular advantage of making it possible to simultaneously record both absorbance and c.d. spectra (and therefore *g*-spectra) of the eluted enantiomers trapped in the chromatographic cell. This allows one to select the most suitable wavelength for monitoring. In Fig. 4 the absorption and circular dichroism spectra of **1** are reported, together with the behaviour of the anisotropy factor in the same spectral region. As far as this case is concerned, if there is a problem with the concentration of the sample, the detection wavelength can be selected in order to give the highest value of the c.d. signal (i.e. around 220 nm). If

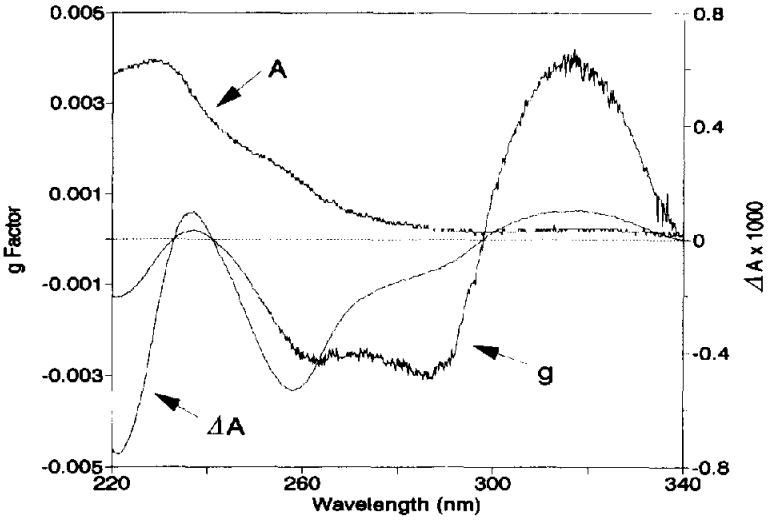

Figure 4
UV (arbitrary scale), c.d. and *g* spectra of a fraction of (*R*)-**1** trapped in the chromatographic cell (for analyte and chromatographic conditions, see Fig. 1).

the highest accuracy is required and there are no limitations on the concentration, as is the usual case in preparative chromatography, the 320 nm wavelength should be selected in order to ensure the highest value of *g*. The possibility of selecting the monitoring wavelength over a very wide range (210–1000 nm) confers on the present detection system a very broad range of applicability in stereochemical analysis by chromatographic methods.

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