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Short Communication

Determination of optical purity by high-performance liquid chromatography upon non-chiral stationary phases with dual circular dichroism/absorption detection

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ABSTRACT

The application of a circular dichroism (CD)-based detection system in HPLC using a non-chiral stationary phase is presented. The simultaneous measurement of CD, absorbance and anisotropy factor allows the determination of optical purity and the assignment of the absolute configuration to the prevailing enantiomer. This represents a further application of CD to analyse an optically active compound. The reliability of the method and the detection limit depend on the value of the anisotropy factor and on the CD intensity, respectively.

INTRODUCTION

The main use of liquid chromatography on chiral stationary phases is to determine the enantiomeric composition of chiral substances by resolution of the chiral analyte [1–5]. Detection systems based on chiroptical properties [6–13] can be profitably used because of their selectivity and because the direct determination of the elution order is often allowed by relating the sign of the observed signal (optical rotation or circular dichroism) to the absolute configuration of the eluates. Further, polarimetric detection has been employed to determine optical purity (o.p.) by HPLC on non-chiral stationary phases [11]. The main advantage of this method is the use of widely available and cheaper columns, even if it presents some limitations. We present here the first application of the CD detection system to determine o.p. by HPLC on non-chiral stationary phases. In particular a silica–diol column was used for the analysis of compounds **1–3** (Fig. 1). A detection system was used that allows the simultaneous monitoring of the CD signal, the absorption and their ratio, *i.e.* the anisotropy factor ($g = \Delta\varepsilon/\varepsilon$) [6,13]. The results obtained show the potential of the method as useful tool in solving problems of stereochemical characterization and quality control of chiral compounds.

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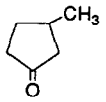

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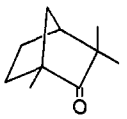

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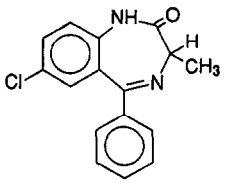

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Fig. 1. Structures of the compounds used: 3-methylcyclopentanone (**1**), fenchone (**2**) and 3-methyl-desmethyldiazepam (**3**).

EXPERIMENTAL

CD detection was carried out by connecting to a Jasco 887-PU HPLC system, incorporating an absorption Multi-340 detector, a Jasco J-500 CD spectrometer equipped with a micro (8 μl volume, 1 cm pathlength) HPLC flow cell. A doublet of lenses was used to focus the light coming from the lamp of the CD spectrometer through the HPLC cell. The J-500 CD spectrometer was modified to detect simultaneously the CD and absorption signals as well as their ratio, *i.e.* the anisotropy factor g [13].

Single enantiomers of **1** and **2** were purchased from Fluka, Switzerland, and Janssen, Belgium, respectively, and (*RS*)-**3** was kindly provided by Professor W.H. Pirkle, University of Illinois, USA. The single enantiomers of (*RS*)-**3** were obtained by HPLC resolution, using an ionic column based on (*R*)-N-(3,5-dinitrobenzoyl) phenylglycine ionically bonded to a 5-μm γ-aminopropyl silanized silica [14]. Hexane–2-propanol (90:10, v/v) was used as mobile phase, at a flow-rate of 1.5 ml/min. The chromatographic analyses of samples of **1–3**, with different enantiomeric composition, were performed by using a Nucleosil-OH column (250 × 4.6 mm I.D., 7 μm) from Machery–Nagel, Düren, Germany. Hexane (HPLC grade) was employed as mobile phase at a flow-rate of 1 ml/min.

RESULTS AND DISCUSSION

Optical purity can be determined by HPLC on non-chiral stationary phases by coupling a chromatographic system to a detector measuring the optical rotation [11]. However, the concentration of the analyte must be independently determined by the UV absorption. The use of two detectors and two independent flow cells is a disadvantage and can limit the reliability of the measurement. Further, the interpretation of the optical rotation is usually more difficult than the analysis of the CD signal. Thus, the simultaneous detection of the CD, the absorption and the anisotropy factor provides a more reliable method to obtain all the information needed for the stereochemical characterization of the sample under investigation. Such a system has been used for the first time by us to analyse chiral samples on non-chiral stationary phases by HPLC. The main advantages are the use of widely available and cheaper columns than the chiral ones, and the easier development of the chromatographic analysis. These aspects make the method a very useful tool in solving stereochemical problems when the chemical purity of the sample is low. In performing the chromatographic analysis, the value of the absorbance gives the concentration of the eluate. The wavelength selected for the monitoring depends of the structure of the analyte. If the molar extinction coefficient (ε) of the sample is not known for the solvent mixture used in the chromatographic analysis, it has to be determined first. The sign and the intensity of the CD signal are related to the absolute configuration of the more abundant enantiomer and to the o.p., respectively. As far as the anisotropy factor is concerned, it is important to stress that its value is independent of the concentration. Thus g is linearly related to the o.p. [o.p. = $(g_{exp}/g_{max}) \times 100$] [6,13] and its value can be used to determine the o.p. directly.

Of course, $\Delta\varepsilon_{max}$ and g_{max} values are needed to determine the o.p., and in several cases the $\Delta\varepsilon_{max}$ values are reported in the literature. When this is not so, or even to check the reliability of

the reported values, $\Delta\varepsilon_{max}$ (l mol^{-1} cm^{-1}), at the wavelength selected for the monitoring, has to be independently determined by a standard.

As an example, in Fig. 2 the chromatographic analysis of **2** is reported. The lower trace was obtained by absorption detection at 292 nm (*i.e.* a wavelength inside the absorption band due to the $n \rightarrow \pi^*$ electronic transition of saturated ketones) and gives the concentration of the analyte. The upper trace was obtained by CD detection at the same wavelength: the sign defines the stereochemistry of the prevailing enantiomer, (+)-**2**, and the value is related to the optical purity (o.p. 100%). Indeed (+)-**2** has a positive CD band at 292 nm, *i.e.* the wavelength selected for monitoring, and the value of the CD signal corresponds to that of the pure (+) enantiomer. In Fig. 3 the linearity plot of the anisotropy factor at 300 nm (a wavelength inside the absorption and CD bands related to the $n \rightarrow \pi^*$ transition) *versus* the percentage of (+)-(*R*)-**1** is reported. Each value represents the average result of three injections. The assay exhibits very good linearity with a correlation coefficient r of 0.9994. In Fig. 4 experimental examples are reported for the analysis of a 100% o.p. sample of (+)-(*R*)-**1** (upper part) and for a 1% o.p. sample of (+)-(*R*)-**1** (lower part). In the latter case the CD and the g signals are still high enough (signal-to-noise $\gg$ 10) to obtain a reliable value. The accuracy of the measure depends of the value of the anisotropy factor. In the case of compound **1** the g value at 300 nm is

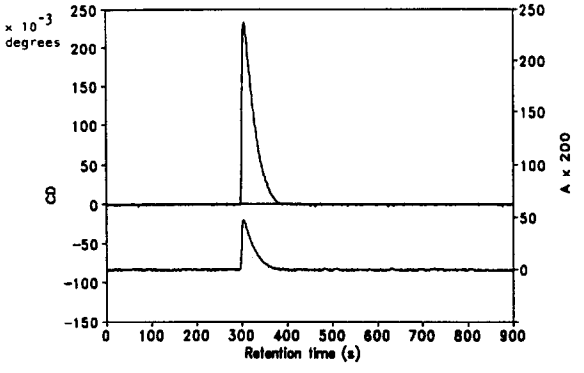

Fig. 2. HPLC analysis of (+)-**2** on a Nucleosil-OH column. Mobile phase hexane at 1 ml/min, monitoring by absorption (lower curve) and CD (upper curve) detection at 292 nm.

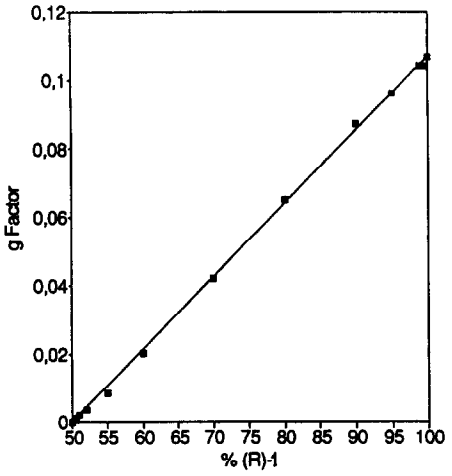

Fig. 3. Linearity plot of g *versus* the percentage of (+)-(*R*)-**1** in the enantiomeric mixtures. The g values represent the average of three analyses monitored at 300 nm.

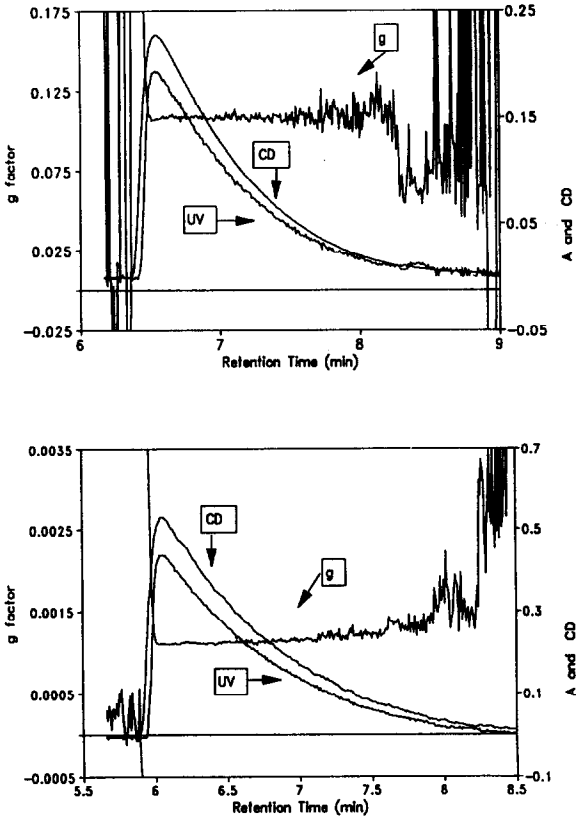

Fig. 4. HPLC analysis of (+)-(*R*)-**1** having 100% o.p. (top) and 1% o.p. (bottom) on a Nucleosil-OH column. Mobile phase hexane at 1 ml/min, monitoring by absorption and CD and g detection at 300 nm. Top: CD × 3 degrees; bottom: CD × 0.03 degrees.

high (>0.1), and thus samples with very low o.p. can be successfully analysed and o.p. down to 0.1% or less reliably determined.

As a further example we analysed compound **2**, which has a relatively high anisotropy factor at 292 nm ($g \sim 0.04$). The linearity of the g value (each value as the average of three runs) at 292 nm with the percentage of (+)-**2** in the enantiomeric mixture was evaluated. Very good linearity was obtained (correlation coefficient $r = 0.9999$). The method is reliably applicable to the analysis of samples of **2** down to o.p. of 0.1% or less.

Finally, in Fig. 5 the experimental chromatographic profile (absorption and CD detection at 254 nm) of a sample of compound (*S*)-**3**, with unknown o.p., is reported. The o.p. of this sample was determined to be 29.0% (wavelength of monitoring 254 nm: CD_{max} 0.0225°; A_{max} 1.148; g_{exp} 0.00059; g_{max} 0.00205), the arithmetric mean of the values obtained by two chromatographic analyses. The wavelength of monitoring was selected on the basis of the CD spectrum of benzodiazepin-2-ones that present a CD maximum at about 254 nm. The sign of the CD at that wavelength has been related to the absolute configuration [13]. It is noteworthy that the reliability of the measurement is still high when, as in this case, the value of the anisotropy factor is of the order of 10^{-3}.

The results discussed above show that the detection system based on the simultaneous measurement of CD, absorption and g factor can be successfully used to determine the o.p. by HPLC on non-chiral stationary phases. This method does not replace HPLC on chiral stationary phases, but is a complementary technique that remarkably increases the number of applications of the CD detector. This system is particularly useful for analysing optically active compounds in complex matrixes (crude reaction products, natural compounds obtained by extraction, drugs in endogenous materials or formulations). The design of the separation required to determine the purity of chiral solutes is indeed much easier taking into account the availability of non-chiral stationary phases whose applicability is well documented.

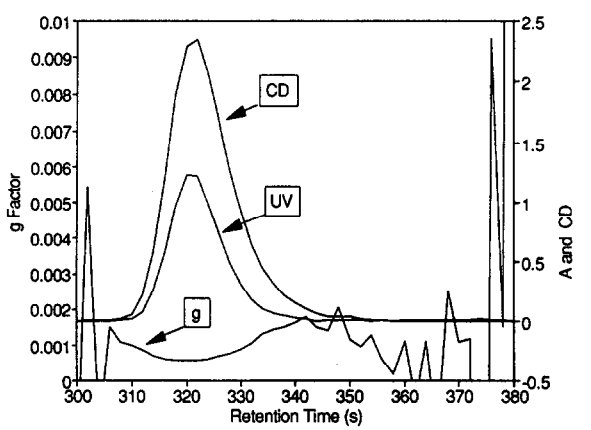

Fig. 5. HPLC analysis of a sample of (*S*)-**3** on a Nucleosil-OH column. Mobile phase hexane at 1 ml/min, monitoring by absorption and CD and g detection at 254 nm. The o.p. was determined to be 29% (details in the text). CD × 10^{-2} degrees.

The detection limit depends of the intensity of the CD signal at the wavelength selected for monitoring (*ca.* 1 μg for a high-o.p. sample of **3**). If the sensitivity represents a limit, the analyte can be derivatized [15] to obtain functionalized derivatives with much higher CD signal (detection limit *ca.* 10 ng for samples with $\Delta\varepsilon > 1000$). The accuracy of the measurement depends on the anisotropy factor of the analysed compound. Samples with o.p. less than 0.5% can be reliably analysed if the g factor is 10^{-3} or larger.

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