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Simultaneous determination of phenylglycidol enantiomers and cinnamyl alcohol in asymmetric epoxidation processes by chiral liquid chromatography☆

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Abstract

A high-performance liquid chromatographic method has been developed for the simultaneous determination of phenylglycidol enantiomers and cinnamyl alcohol (CA). Separations were achieved on an amylose tris(3, 5-dymethylphenylcarbamate) chiral stationary phase (Chiralpak AD). The effect of concentration of organic modifier (2-propanol and ethanol) in the mobile phase and flow-rate was studied. The mobile phase selected consisted of a mixture of *n*-hexane–ethanol (85:15, v/v) with a flow-rate of 1.2 ml/min. The UV–vis detector was set at 254 nm. Resolution for the phenylglycidol enantiomers in the suitable chromatographic conditions was 2.4 with an analysis time of 12 min. The method developed was validated and was found to be linear in the range from 5×10^{-4} to 3×10^{-2} M, for phenylglycidol enantiomers and in the range from 5×10^{-5} to 1×10^{-3} M, for CA ($r > 0.999$ for the three compounds). Repeatability and intermediate precision for the three analytes at three different concentrations were below 3.6 and 2.8% R.S.D., respectively. This method has been applied to study the asymmetric epoxidation of CA with titanium(IV) alkoxide compounds as catalysts in order to evaluate their catalytic activity and stereoselectivity of the epoxidation processes.

Keywords: Enantiomer separation; Epoxidation, asymmetric; Phenylglycidol; Cinnamyl alcohol; Alcohols

1. Introduction

The synthesis of enantiomerically pure compounds is of great importance not only in organic chemistry as well as medical and agricultural chemistry, but also in the pharmaceutical and agricultural industries. Over the past two decades, asymmetric synthesis has undergone a rapid expansion as a tool in obtaining enantiomerically pure compounds, and it appears increasingly to be the future method for the production of these compounds [1].

Since, the discovery of asymmetric epoxidation in 1980 by Sharpless-Katsiki [2], catalytic asymmetric epoxidation of allylic alcohols is a powerful strategy in the synthesis of enantiomerically enriched epoxides [3]. New catalytically active species are synthesised continuously for this kind of processes [4] and some methods have been developed to evaluate the enantiomeric purity of reagents used in asymmetric synthesis as catalyst, auxiliaries and synthons [5]. However, in order to evaluate the catalytic activity and stereoselectivity of these compounds, it is of great importance also to develop simple and rapid validated analytical methods for routine work [6].

The most common methods for screening asymmetric reactions involve chiral chromatography because it is highly desirable to observe both, educt and product simultaneously in screening procedures [7–10]. Chiral HPLC and GC have been used because of their accuracy in determining enantiomeric

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excess (e.e.). However, analytical methods for screening asymmetric epoxidation reactions are scarce, and as far as we know, no article has been published in available literature on analysis by chiral HPLC of cinnamyl alcohol (CA) and phenylglycidol enantiomers in these types of samples.

In this work, we have developed and validated a method for the separation by chiral liquid chromatography of phenylglycidol enantiomers and CA. This method was applied in the determination of e.e. and yield obtained in asymmetric epoxidation processes of CA with different chiral titanium complexes as catalysts, previously synthesized in our laboratory.

2. Experimental

2.1. Chemicals and reagents

Synthesis of chiral titanium complexes $Ti(O^iPr)_2(OR)_2$ (**1**, **2**, **3**, **4**; Fig. 1) has been reported previously [4]. 3-Phenyl-2-propenol (CA) 98% and (2S, 3S)-(−)-3-phenylglycidol 97% [(S,S)-PG] were purchased from Sigma–Aldrich (Alcobendas, Spain). (2R, 3R)-(+)-3-phenylglycidol 96% [(R,R)-PG] was purchased from Acros Organics (Geel, Belgium). HPLC-grade *n*-hexane, 2-propanol, ethanol and methanol were obtained from Merck (Darmstadt, Germany). tert-Buthyl hydroperoxide (TBHP) 5.0–6.0 M solution in nonane, powdered 4 Å molecular sieves and Celite were purchased from Sigma-Aldrich. Sodium hydroxide synthesis grade and magnesium sulphate anhydrous extra pure were obtained from Sharlau (Barcelona, Spain). Sodium chloride synthesis grade was purchased from Panreac (Barcelona, Spain). Dichloromethane and diethyl ether synthesis grade were obtained from SDS (Barcelona, Spain).

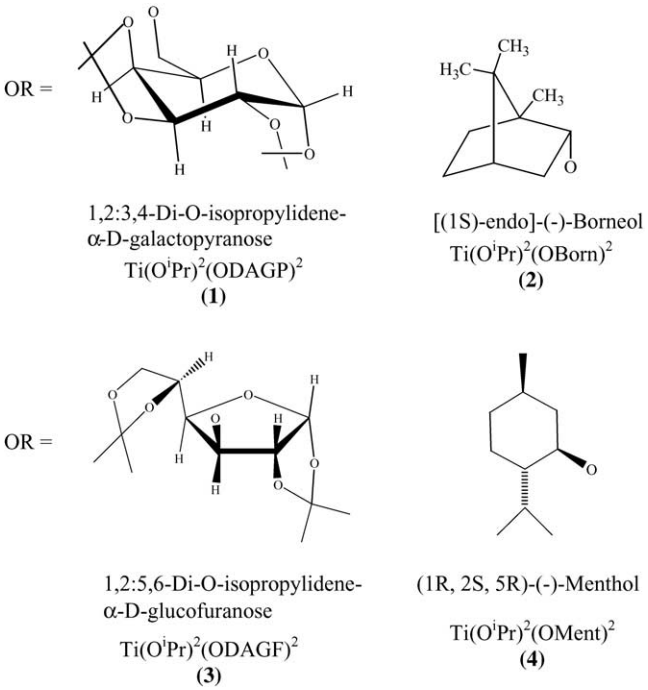

Fig. 1. Structures of $[Ti(O^iPr)_2(OR)_2]$ catalysts employed for the epoxidation of cinnamyl alcohol.

2.2. Apparatus

HPLC analyses were performed on a Varian chromatographic system containing a 210/215 ProStar pump, a manual injection valve Rheodyne model 7725i equipped with a 20 μl sample loop (Rheodyne, Cotati, CA, USA), a 320 ProStar UV–vis detector and a personal computer-based data acquisition system Star Chromatography Workstation version 5.

UV–vis spectra of the eluted peaks were achieved in a Varian UV–vis spectrophotometer CARY 50conc.

2.3. Chromatographic conditions

The chromatographic separations were performed, using a Chiralpak AD [amylose tris(3,5-dymethylphenylcarbamate) coated on silicagel, 250 mm × 4.6 mm i.d., 10 μm particle diameter] column (Chiral Technologies Europe, Illkirch, France) at ambient temperature. The mobile phase selected for the method validation and for the determination of the e.e. of phenylglycidol enantioners obtained after catalytic asymmetric epoxidation of CA consisted of a mixture of *n*-hexane–ethanol (85:15, v/v) delivered at a flow-rate of 1.2 ml/min. Before use, the mobile phase was degassed for 15 min in an ultrasonic bath. The samples were monitored with UV detection at 254 nm.

2.4. Standard solutions

The appropriate amount of CA, (R,R)-PG and (S,S)-PG was dissolved into methanol to give stock solutions with a final concentration of 0.1 mol l^{-1}.

Six solutions were prepared daily fresh by diluting each stock solution with mobile phase to achieve concentrations ranging from 5×10^{-5} to 1×10^{-3} M for the CA and from 5×10^{-4} to 3×10^{-2} M for the phenylglycidol enantiomers.

2.5. Sample preparation

A flame dried 250 ml two-necked flask was fitted dropping funnel and flushed with nitrogen, and charged with 2 g of activated, powdered 4 Å molecular sieves 0.3–0.7 g of catalyst $Ti(O^iPr)_2(OR)_2$ (**1**, **2**, **3** or **4**; Fig. 1) and 100 ml of dry dichloromethane. Then the mixture was cooled at −20 °C and 0.3–1.0 ml of a 5.5 M solution of TBHP in nonane was added. The mixture was allowed to stir at −20 °C for 1 h and then treated with 1–3 ml of a 0.37 M solution of freshly distilled CA in dichloromethane, added drop wise over 1 h. The resulting homogeneous solution was stored for 5 h at −20 °C. The reaction mixture was quenched with 0.4 ml of a 10% (w/v) aqueous solution of sodium hydroxide saturated with sodium chloride. The cold bath was removed and the mix-

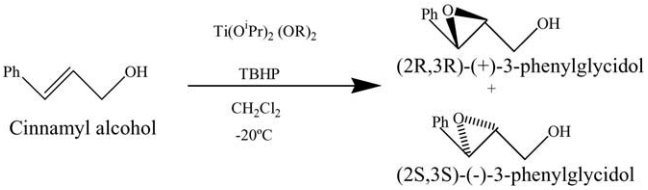

Fig. 2. Scheme of asymmetric epoxidation processes of cinnamyl alcohol with chiral titanium complexes as catalysts.

ture was stirred for 10 min. Then the mixture was treated with magnesium sulphate anhydrous and Celite, and after the solution was filtered, washing with diethyl ether. The volatiles were removed in vacuum getting yellow oil that was reconstituted in 2–10 ml of ethanol and filtered through a 0.45 μm pore size nylon filter membrane (Fig. 2).

3. Results and discussion

3.1. Optimization of chiral separation conditions

Optimization of the chiral separation of CA and phenylglycidol enantiomers on the Chiralpak AD column was done with respect to the chosen optimization criteria: resolution, R_s, should be higher than 1.5, analysis time should not exceed 15 min and flow-rate of the mobile phase should not exceed 1.2 ml/min. The main parameters taken into consideration for improving chiral separations on the Chiralpak AD column were the type and concentration of the organic modifier in the mobile phase. When the organic modifier in the mobile phase was 2-propanol, attempts failed to separate CA and phenylglycidol enantiomers on the Chiralpak AD column (Table 1, Fig. 3). Better results were obtained by changing the polar organic modifier from 2-propanol to ethanol and optimizing their concentration and mobile phase flow-rate (Table 2). When the mobile phase consisted in a mixture *n*-hexane–ethanol (90:10, v/v), the increase in the flow-rate from 0.8 to 1.2 ml/min reduced the analysis time from 25 to 17 min. By changing the proportion of *n*-hexane–ethanol from (90:10, v/v) to (85:15, v/v), at a flow-rate of 1.2 ml/min, the value of the resolution for both enantiomers, $R_{s(SS/RR)}$, decreased from 2.7 to 2.4, but the duration of analysis also

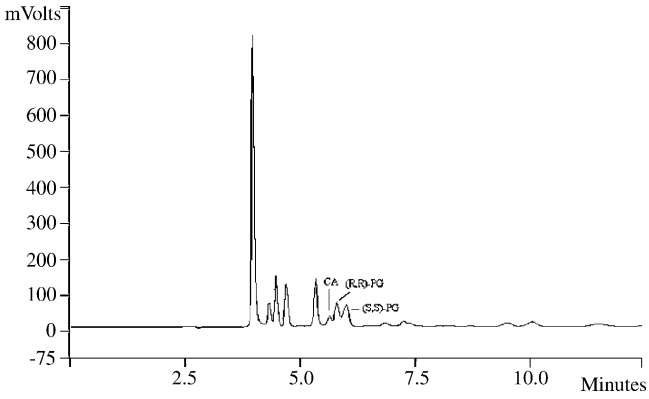

Fig. 3. Separation of cinnamyl alcohol (CA) and phenylglycidol enantiomers [(R,R)-PG and (S,S)-PG] using 2-propanol as polar modifier in *n*-hexane. Chromatographic conditions: column, Chiralpak AD 250 mm × 4.6 mm i.d. 10 μm; mobile phase, *n*-hexane–2-propanol (80:20, v/v); flow-rate 1 ml/min; column temperature, ambient; detection, 254 nm.

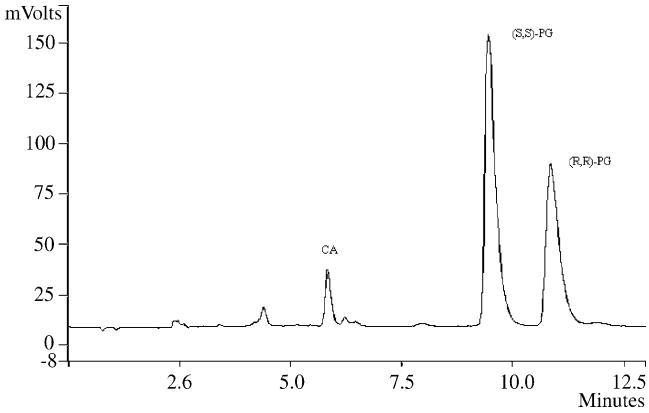

Fig. 4. Separation of cinnamyl alcohol (CA) and phenylglycidol enantiomers [(R,R)-PG and (S,S)-PG] using optimised conditions. Chromatographic conditions: column, Chiralpak AD 250 mm × 4.6 mm i.d. 10 μm; mobile phase, *n*-hexane–ethanol (85:15, v/v); flow-rate 1.2 ml/min; column temperature, ambient; detection, 254 nm.

decreased from 17 to 12 min. Thus, the mobile phase *n*-hexane–ethanol (85:15, v/v) at a flow-rate of 1.2 ml/min was chosen as a compromise between the analysis time and resolution. As it can be see in Fig. 4, the mobile phase *n*-hexane–ethanol (85:15, v/v) at a flow-rate of 1.2 ml/min al-

Table 1
Summary of chromatographic parameters for cinnamyl alcohol and phenylglycidol enantiomers separation on a Chiralpak AD column using 2-propanol as organic modifier in *n*-hexane at different flow-rates

Mobile phase: *n*-hexane–2-propanol	Flow-rate (ml/min)	R_s		k'				α	t_a (min)
		CA/(R,R)-PG	(R,R)-PG/(S,S)-PG	CA	(R,R)-PG	(S,S)-PG	(R,R)-/(S,S)-PG		
95:5 (v/v)	0.7	–	0.5	–	4.26	4.42	1.04	23	
	0.8	–	1.0	–	4.26	4.45	1.04	20	
90:10 (v/v)	0.8	0.9	0.8	1.16	1.24	1.31	1.06	12	
	1.0	1.1	0.9	1.16	1.24	1.32	1.06	10	
80:20 (v/v)	1	0.7	0.7	1.25	1.31	1.40	1.06	7	

CA, cinnamyl alcohol; (R,R)-PG, (2R,3R)-(+)-3-phenylglycidol; (S,S)-PG, (2S,3S)-(−)-3-phenylglycidol; R_s, resolution; k', retention factor; α, selectivity; t_a, time of analysis; (–), not calculated.

Table 2
Summary of chromatographic parameters for cinnamyl alcohol and phenylglycidol enantiomers separation on a Chiralpak AD column using ethanol as organic modifier in *n*-hexane at different flow-rates

Mobile phase: *n*-hexane–ethanol	Flow-rate (ml/min)	R_s		k'			α	t_a (min)
		CA-/(S,S)-PG	(S,S)-PG/(R,R)-PG	CA	(S,S)-PG	(R,R)-PG	(S,S)-/(R,R)-PG	
90:10 (v/v)	0.8	7.9	2.4	2.05	4.30	5.18	1.21	25
	1.0	9.6	2.7	2.06	4.30	5.19	1.21	20
	1.2	9.6	2.7	2.05	4.29	5.17	1.20	17
85:15 (v/v)	1.2	8.4	2.4	1.45	2.97	3.60	1.20	12

CA, cinnamyl alcohol; (R,R)-PG, (2R,3R)-(+)-3-phenylglycidol; (S,S)-PG, (2S,3S)-(−)-3-phenylglycidol; R_s, resolution; k', retention factor; α, selectivity; t_a, time of analysis.

lowed the baseline separation of phenylglycidol enantiomers. The CA eluted at a retention time of 5.8 min with a retention factor (k') of 1.45 (calculated with a dead time of 2.4 min from the first disturbance of the baseline), the first enantiomer (S,S)-PG eluted at a retention time of 9.4 min (k' = 2.97) and the second enantiomer (R,R)-PG eluted at a retention time of 10.8 min (k' = 3.60).

3.2. Method validation

3.2.1. Specificity

The specificity of the method developed was tested towards the main compounds that could be present in the samples after the asymmetric epoxidation of the CA, such as TBHP and dichloromethane. The injection of these potential interfering compounds in the chromatograph demonstrated their absence at the retention times of the phenylglycidol enantiomers and CA.

For purity control of the phenylglycidol peaks in the samples, UV-vis absorption spectra of the eluted peaks was obtained and compared with absorption spectra of standard solutions of the phenylglycidol enantiomers (Fig. 5).

3.2.2. Linearity

Six standard solutions at different concentration levels ranging from 5×10^{-4} to 3×10^{-2} M for both phenylglycidol enantiomers and from 5×10^{-5} to 1×10^{-3} M for the CA were prepared and checked for linearity. The linear regression analysis was made by plotting peaks areas (y) versus analyte concentrations (x) in M.

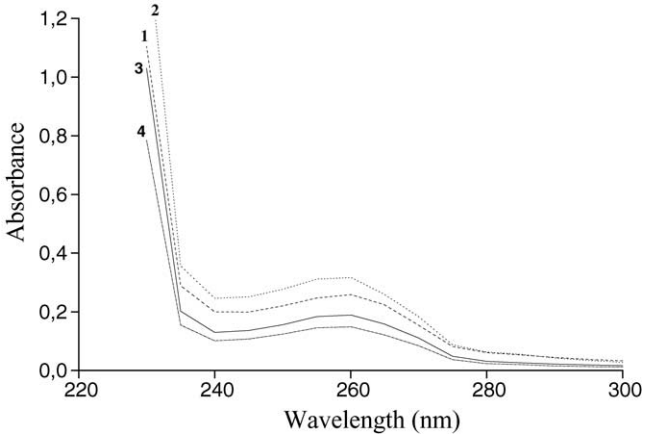

Fig. 5. Absorption spectra of the (a) (R, R)- and (b) (S, S)- phenylglycidol peaks in a epoxidation sample and (c) (R, R)- and (d) (S, S)- phenylglycidol peaks in standard solutions.

Equation, determination coefficient (r^2) and R.S.D. value (%) for the slope and intercept of the calibration curves are indicated in Table 3. The determination coefficient of the plots obtained for both enantiomers and CA (more than 0.999) demonstrates good linearity.

3.2.3. Detectability

The limits of detection (LOD) and quantification (LOQ) were determined as the concentrations of analyte giving rise to signal-to-noise ratios (S/N) of 3 and 10, respectively. The LODs for (R,R)- and (S,S)-PG enantiomers and CA were found to be 1.0×10^{-4}, 1.6×10^{-4} and 5.5×10^{-4} M, respectively, and the LOQs were found to be 4.9×10^{-4}, 5.0×10^{-4} and 3.8×10^{-5} M, respectively (Table 3).

3.2.4. Precision

Precision of the method was studied for repeatability and intermediate precision. Assay values were obtained for the three analytes of interest at three concentrations levels (5×10^{-4}, 5×10^{-3} and 3×10^{-2} M for both enantiomers, and 5×10^{-5}, 4×10^{-4} and 1×10^{-3} M for CA). Repeatability was expressed in terms of R.S.D. values (%) calculated from 1-day data (n = 6, k = 1). R.S.D. values were found to be below 3.6%, indicating a good repeatability. Intermediate precision was calculated for each concentration level from three consecutive days data (n = 9, k = 3) and expressed in terms of R.S.D. values (%). At each concentration levels the R.S.D. values were below 2.8%, indicating a good intermediate precision (Table 3).

3.2.5. Recovery

Recovery experiments were conducted to determine the accuracy of the method for the quantification of phenylglycidol enantiomers present in the samples. Accuracy of the method was checked at 5×10^{-3} M in triplicate. The overall accuracy of the procedure was assessed by comparing the amount of enantiomers found in a sample versus the amount of enantiomers found in the same spiked sample. Recovery of the (R,R)-PG and (S,S)-PG was with an average of

Table 3
Validation parameters of the chiral method used in the determination of phenylglycidol enantiomers and cinnamyl alcohol

Validation parameter	(2R, 3R)-(+)-3-phenylglycidol	(2S, 3S)-(−)-3-phenylglycidol	Cinnamyl alcohol
Linearity (n = 6)	$y = 135827x - 57.77$	$y = 195236x - 41.21$	$y = 2.19 \times 10^7 x + 183$
Concentration (M)	$5 \times 10^{-4} - 3 \times 10^{-2}$	$5 \times 10^{-4} - 3 \times 10^{-2}$	$5 \times 10^{-5} - 1 \times 10^{-3}$
r^2	0.9990	0.9996	0.9998
R.S.D.$_{slope}$ (%)	8.5	8.8	8.5
R.S.D.$_{intercept}$ (%)	9.6	19	6.6
LOD (M)	1.0×10^{-4}	1.6×10^{-4}	5.5×10^{-4}
LOQ (M)	4.9×10^{-4}	5.0×10^{-4}	3.8×10^{-5}
Repeatability (n = 6, k = 1) M (% R.S.D.)			
5×10^{-4}	2.3	3.0	–
5×10^{-3}	3.6	2.6	–
3×10^{-2}	0.6	0.8	–
5×10^{-5}	–	–	0.7
4×10^{-4}	–	–	0.5
1×10^{-3}	–	–	0.6
Intermediate precision (n = 9, k = 3) M (% R.S.D.)			
5×10^{-4}	1.5	2.8	–
5×10^{-3}	0.8	1.0	–
3×10^{-2}	1.1	0.6	–
5×10^{-5}	–	–	0.8
4×10^{-4}	–	–	0.6
1×10^{-3}	–	–	0.4
Accuracy (n = 3)			
%Recovery	99.5 ± 0.3	100.1 ± 0.6	–
%R.S.D.	0.2	0.6	–

Chromatographic conditions: column, Chiralpak AD 250 mm × 4.6 mm i.d. 10 μm; mobile phase, *n*-hexane–ethanol (85:15, v/v); flow-rate 1.2 ml/min; column temperature, ambient; detection, 254 nm; (–), not calculated; k = number of days.

99.5 and 100.1%, respectively, with an R.S.D. below 0.6% (Table 3).

3.3. Application of the method

The method developed has been applied successfully for routine analysis of phenylglycidol enantiomers after the catalytic asymmetric epoxidation of CA with different chiral titanium complexes synthesised in our laboratory [4] as catalysts. Results obtained in terms of e.e. and yield for the catalytic asymmetric epoxidation of CA with chiral titanium complexes **1**, **2**, **3** and **4** (Fig. 1) under different catalyst/substrate ratio (1/1, 1/2, and 1/20 mol/mol) are indicated in Table 4. The results obtained show that the e.e. increase by decreasing the catalyst/substrate ratio but, the yield obtained in the epoxidation processes also decrease. The e.e. obtained, with catalysts **2** and **3**, in the (1/1) catalyst/substrate ratio was more than 50% which indicated a good catalytic activity of these catalysts. Fig. 6 shows a chromatogram of a sample obtained after epoxidation with **3**.

Table 4
Enantiomeric excess and yield for the catalytic asymmetric epoxidation of cinnamyl alcohol with chiral titanium complexes under different catalyst/substrate ratio

Catalyst[a]	Catalyst/substrate ratio (mol/mol)					
	1/1		1/2		1/20	
	e.e.[b] (%)	Yield (%)	e.e. (%)	Yield (%)	e.e. (%)	Yield (%)
$Ti(O^iPr)_2(ODAGP)_2$	33	13	24	29	15	57
$Ti(O^iPr)_2(ODAGF)_2$	60.5	11	19	19	16.5	56.5
$Ti(O^iPr)_2(OMent)_2$	58	13	23	30	13	59
$Ti(O^iPr)_2(OBorn)_2$	43	13	25	28	15	59

[a] See Fig. 1.

[b] e.e. = Enantiomeric excess = {[(R,R)-PG] − [(S,S)-PG]/[(R,R)-PG] + [(S,S)-PG]} × 100 (R,R)-PG = (2R,3R)-(+)-3-phenylglycidol; (S,S)-PG = (2S,3S)-(−)-3-phenylglycidol Chromatographic conditions: column, Chiralpak AD 250 mm × 4.6 mm i.d. 10 μm; mobile phase, *n*-hexane–ethanol (85:15, v/v); flow-rate 1.2 ml/min; column temperature, ambient; detection, 254 nm.

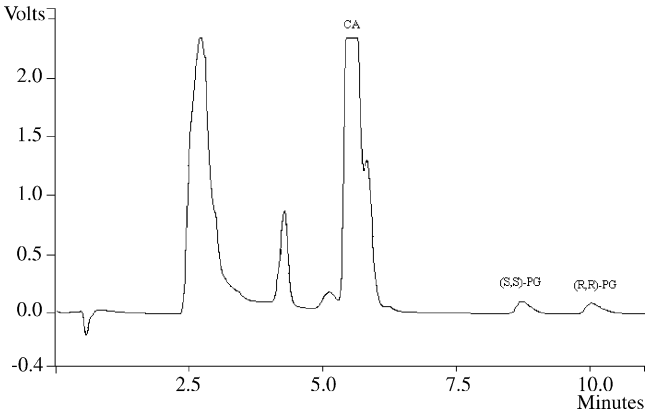

Fig. 6. Representative chromatogram of a cinnamyl alcohol epoxidation sample with $Ti(O^iPr)_2(ODAGF)_2$ as catalyst (catalyst/substrate ratio = 1/20 mol/mol) using optimised conditions. CA, cinnamyl alcohol; (S,S)-PG, (2S,3S)-(−)-3-phenylglycidol; (R,R)-PG, (2R,3R)-(+)-3-phenylglycidol. Chromatographic conditions: column, Chiralpak AD 250 mm × 4.6 mm i.d. 10 μm; mobile phase, *n*-hexane–ethanol (85:15, v/v); flow-rate 1.2 ml/min; column temperature, ambient; detection, 254 nm.

4. Conclusions

A chiral HPLC method has been developed and validated for the simultaneous determination of CA and phenylglycidol enantiomers, using a Chiralpak AD column. The separation of two enantiomers was obtained with adequate resolution (R_s = 2.4) and selectivity using a mobile phase *n*-hexane–ethanol (85:15, v/v) at a flow-rate of 1.2 ml/min. The method is simple, selective, precise and accurate, and it is useful for screening asymmetric epoxidation processes of CA with chiral titanium complexes as catalysts.

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