Comparative Evaluation of Four Detectors in the High-Performance Liquid Chromatographic Analysis of Chiral Nonaromatic Alcohols

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Abstract

A comparative evaluation of ultraviolet, polarimetric, refractive index, and evaporative light-scattering detection coupled with high-performance liquid chromatography has been developed for the separation and quantitation of the enantiomers of chiral nonaromatic alcohols, some of which are intermediates in the synthesis of chiral drugs. (*R,S*)-3-*tert*-butylamino-1,2-propanediol; (*R,S*)-glycidol; and (*R,S*)-1-(4-morpholino)-2-octanol are selected as model compounds in order to compare the detection sensitivity and the linearity of the response with the four detectors. Separation of the enantiomers is performed using chiral stationary phases in normal-phase liquid chromatography. A one-day validation is achieved for (*S*)-3-*tert*-butylamino-1,2-propanediol with each detector, and limits of quantitation are determined for the three compounds. Advantages and limitations of the four detectors are discussed.

Introduction

As is well-known, enantiomeric pairs give different pharmacological responses and can even have differences in toxicity profiles (e.g., thalidomide). Therefore, the increasing production of enantiomerically pure drug compounds in the pharmaceutical industry imposes the development of sensitive methods for enantiomeric purity control. High-performance liquid chromatography (HPLC) is most often used, either with chiral or achiral column material. In achiral column systems, the coeluting enantiomers can only be discriminated by using a chiroptical detector such as a polarimeter (1–5). Although somewhat selective, the sensitivity of this detector depends on the rotatory power of the analyte. However, in chiral HPLC systems, enantiomers elute separately because of the in situ formation of labile diastereoisomeric complexes. Achiral detection modes such as ultraviolet (UV) detection can be used in this case. Therefore, chiral HPLC systems coupled with achiral detection modes are used preferentially in the analytical field (6). However, for the analysis of chromophoric chiral compounds such as some intermediates in the synthesis of chiral drug compounds, alternative solutions to UV detection have to be found. Evaporative light-scattering detection (ELSD) represents a universal detection mode suitable for any sample appreciably less volatile than the mobile phase, whatever the optical properties of the analyte and the mobile phase may be (7–13). In the same manner, the refractive index (RI) detector is universal, but it requires highly standardized chromatographic conditions and is incompatible with gradient elution (7,14–16).

Three nonaromatic chiral alcohols were selected in order to compare the potentialities of UV, ELSD, polarimetric, and RI detection coupled with HPLC for the quantitation of their enantiomers. The chemical structures are illustrated in Figure 1. The
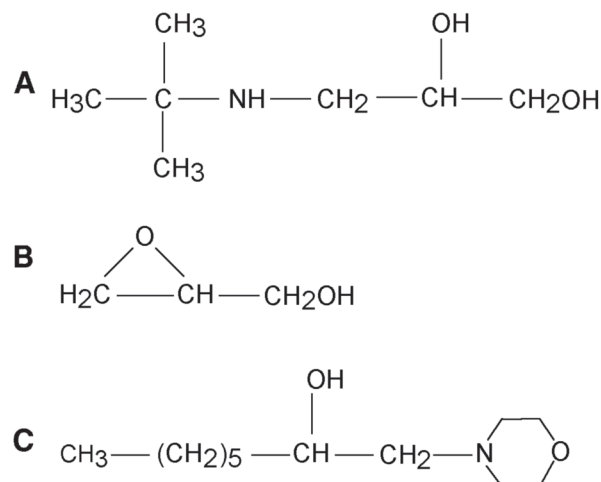
Figure 1. Chemical structures of 3-*tert*-butylamino-1,2-propanediol, A; glycidol, B; and 1-(4-morpholino)-2-octanol, C.

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first compound (3-*tert*-butylamino-1,2-propanediol) is an amino alcohol that shows low absorption properties in the UV region. An HPLC–ELSD method has been previously developed for the enantioseparation and the quantitation of this compound (17).

In this study, the limit of quantitation (LOQ) and the linearity of 3-*tert*-butylamino-1,2-propanediol response were investigated using UV, polarimetric, and RI detection and were compared to ELSD. In addition, the enantiomers of two other nonchromophoric compounds—glycidol (an aliphatic epoxide) and 1-(4-morpholino)-2-octanol (a dialkylamino alcohol)—were separated by HPLC and quantitated using the four detection modes. LOQs were determined and compared.

Advantages and disadvantages of ELSD, UV, polarimetry, and RI in quantitative studies were also evaluated as a function of the chemical and physical properties of the analyte.

Experimental

Chemicals and reagents

(*R*,*S*)-3-*tert*-butylamino-1,2-propanediol and (*S*)-3-*tert*-butyl-amino-1,2-propanediol were kindly offered by DSM Fine Chemicals (Venlo, The Netherlands). (*R*,*S*)-glycidol and (*R*)-glycidol were provided by DSM Research (Geleen, The Netherlands). (*R*,*S*)-1,2-epoxyoctane, (*R*)-1,2-epoxyoctane, and morpholine were from Aldrich (Sigma-Aldrich, Zwijndrecht, The Netherlands). Ethanol, *n*-hexane, and formic acid (98–100%) were of analytical grade and were obtained from Merck (Darmstadt, Germany). Diethylamine (99%) was obtained from Fluka.

Instrumentation and methods

Chromatographic system

The same HPLC system was used throughout the study and coupled successively with the four different detectors. This system was a model HP-1100 liquid chromatograph (LC) equipped with a quaternary pump from Hewlett-Packard (Palo Alto, CA). It was combined with an autosampler Midas from Spark Holland (Emmen, The Netherlands). The HPLC separations were performed at 25°C using a 25-µL injection loop. Quantitation was performed with a LabSystems Xchrom data system (Manchester, UK). The enantioseparation of 3-*tert*-butylamino-1,2-propanediol was achieved using a Chiralpak AS column (10 µm, 250- × 4.6-mm i.d.). The enantioseparation of glycidol and 1-(4-morpholino)-2-octanol were obtained with a Chiralpak AD column (10 µm, 250- × 4.6-mm i.d.). All columns were obtained from Merck (Amsterdam, The Netherlands). The enantiomers of 3-*tert*-butylamino-1,2-propanediol were separated using a mixture of *n*-hexane, ethanol, formic acid, and diethylamine (90:10:0.2:0.2, v/v/v/v) as a mobile phase. The mobile phases used for the enantioseparation of glycidol and 1-(4-morpholino)-2-octanol were composed of *n*-hexane and ethanol (90:10, v/v) and (95:5, v/v), respectively. The mobile phases were degassed for 10 min in an ultrasonic bath before use. The flow rate was 1.0 mL/min.
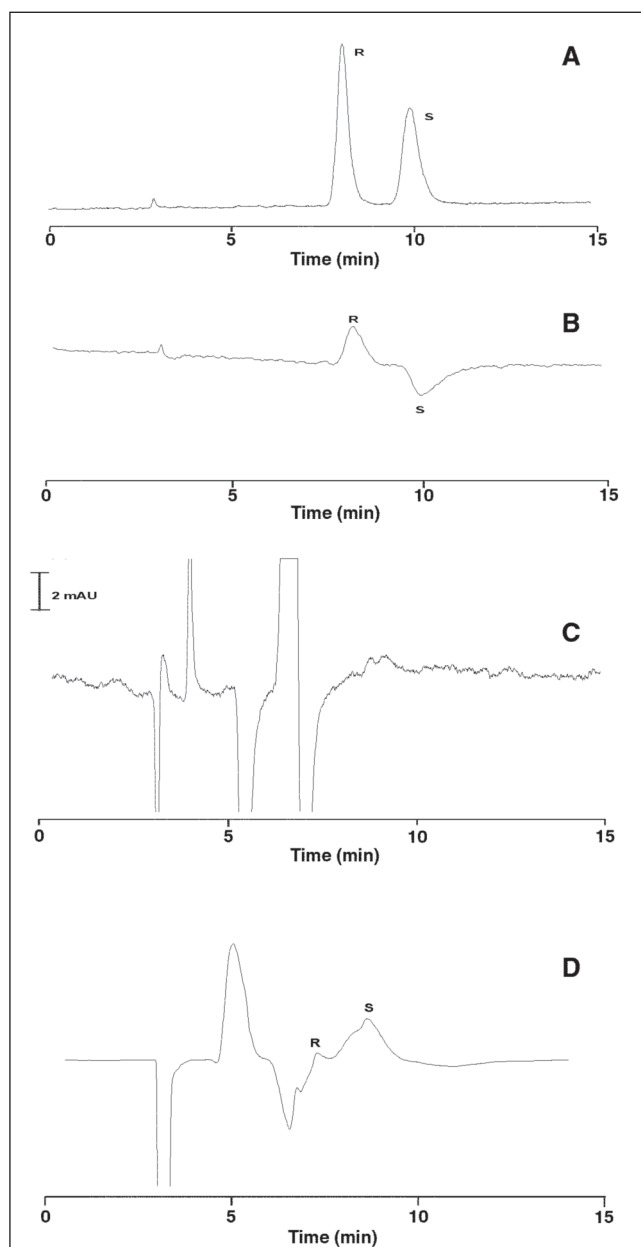
Detection

ELSD was achieved with a Sedex 55 model from S.E.D.E.R.E. (Alfortville, France). The siphon of the waste tube was filled with water purified with a Milli-Q system (Millipore, Bedford, MA). The evaporation gas was helium. The helium pressure and the drift tube temperature were optimized for each analyte. The gain was set to level 5.

UV detection was achieved at 210 nm with a Spectrasystem UV2000 from Thermo Separation Products (Fremont, CA).

The polarimeter was an IBZ (Hannover, Germany) model Chiralyser detector. The average and the range were set to level 10 and 256, respectively.

The RI detector was a Shodex RI-71 from Showa Denko (Tokyo, Japan). The detection was performed at 25°C. The instrument was set to fast-response mode, negative polarity, and a range of 512 was selected.

Figure 2. Enantioseparation of racemic 3-*tert*-butylamino-1,2-propanediol in ELSD (2 mg/mL), A; in polarimetric detection (1 mg/mL), B; in UV (3 mg/mL), C; and in RI (5 mg/mL), D.

Sample solutions

Using the ELSD, (*R,S*)- and (*R*)-3-*tert*-butylamino-1,2-propanediol were dissolved in the mobile phase. However, for UV, polarimetric, and RI detection, 3-*tert*-butylamino-1,2-propanediol was dissolved in a mixture of *n*-hexane, ethanol, formic acid, and diethylamine (60:40:0.2:0.2, v/v/v/v) in order to ensure complete dissolution of 3-*tert*-butylamino-1,2-propanediol solubility at higher concentrations.

(*R,S*)- and (*R*)-glycidol were dissolved in a mixture of *n*-hexane and ethanol (60:40, v/v).

(*R,S*)- and (*R*)-1-(4-morpholino)-2-octanol were synthetized according to the procedure that was reported in literature by Harris et al. (18). (*R,S*)-1,2-epoxyoctane (1.28 g, 10 mmol) and morpholine (2 g, 23 mmol) were mixed and heated in an oven at 80°C for 4 h. The resulting solution was used as a stock solution of 1-(4-morpholino)-2-octanol (555.6 mg/mL) for further analysis. The (*R*)-enantiomer was synthetized in the same way using (*R*)-1,2-epoxyoctane. Sample solutions of (*R,S*)- and (*R*)-1-(4-morpholino)-2-octanol were obtained by diluting the stock solution with the mobile phase.

Results and Discussion

HPLC–ELSD
*3-*tert*-Butylamino-1,2-propanediol*

3-*tert*-Butylamino-1,2-propanediol enantiomers were separated in LC with a resolution (Rs) of 2.65 and detected in ELSD at 45°C and 3.3 bar as helium pressure (17). A boiling point of 265°C (at 1 atm) for 3-*tert*-butylamino-1,2-propanediol made this compound easily detectable in ELSD using a normal-phase LC method.

LOQs were determined as the concentration of substance giving a signal-to-noise ratio of 10. The LOQs of (*R*)- and (*S*)-3-*tert*-butylamino-1,2-propanediol by ELSD were found to be 26 and 48 µg/mL, respectively, which corresponds to the injection of 0.7 and 1.2 µg of (*R*)- and (*S*)-3-*tert*-butylamino-1,2-propanediol, respectively. Figure 2A illustrates the enantioseparation of racemic 3-*tert*-butylamino-1,2-propanediol at 2 mg/mL. (*S*)- and (*R*)-3-*tert*-butylamino-1,2-propanediol areas were significantly different at equal concentrations.

The results of the one-day validation for (*S*)-3-*tert*-butylamino-1,2-propanediol are reported in Table I (17). Six independent injections (*n* = 6) were performed at extreme and middle-scale concentration levels, whereas *n* was equal to 3 at other concentrations. The (*R*)-enantiomer was used at 50 µg/mL as an internal standard for the quantitation of the (*S*)-form. As often reported in literature (10,19–21), ELSD was found to be a nonlinear detection mode. The exponential curve obtained by plotting the peaks's area ratio (*y*) versus the (*S*)-/(*R*)-3-*tert*-butylamino-1,2-propanediol concentration in µg/mL (*x*) could be transformed into a curve corresponding to a second-order polynomial equation by a logarithmic transformation of the data (r^2 = 0.9996). It has to be noted that a linear model could be used within a restricted range of concentration from 500 to 1000 µg/mL. However, the use of the polynomial model allowed for the quantitation of samples in the whole concentration range of 50–1500 µg/mL corresponding to

an enantiomeric excess range of 0–94%. Therefore, this model was more suitable for enantiomeric purity control.

Method accuracy was determined by plotting the graph of the amount of (*S*)-3-*tert*-butylamino-1,2-propanediol found versus the amount applied in samples at three concentration levels (*n* = 6). According to the validation guidelines reported in literature (22–23), the tested procedure could be considered accurate with recoveries included in the interval 98–102% and a mean recovery of 100.1% with a confidence interval (CI) of 1.1%.

Satisfactory results were obtained for repeatability at three concentration levels (*n* = 6) and are reported in Table I.

Glycidol

(*R*)- and (*S*)-glycidol were separated according to a method described elsewhere (24). An Rs of 2.1 was obtained.

Because of its relatively low boiling point (166°C at 1 atm), glycidol was partially vaporized in ELSD (even at 25°C), which resulted in very low detection sensitivity. Using 0.4 bar as the optimal helium pressure, the LOQ was 19.5 mg/mL for both enantiomers. Figure 3A illustrates the enantioseparation of racemic glycidol at 50 mg/mL. The results give an indication of the minimal volatility that is required for a compound to be analyzed by ELSD with a given HPLC mobile phase. In addition, no

Table I. Validation Results for (*S*)-3-*tert*-Butylamino-1,2-Propanediol Using HPLC–ELSD
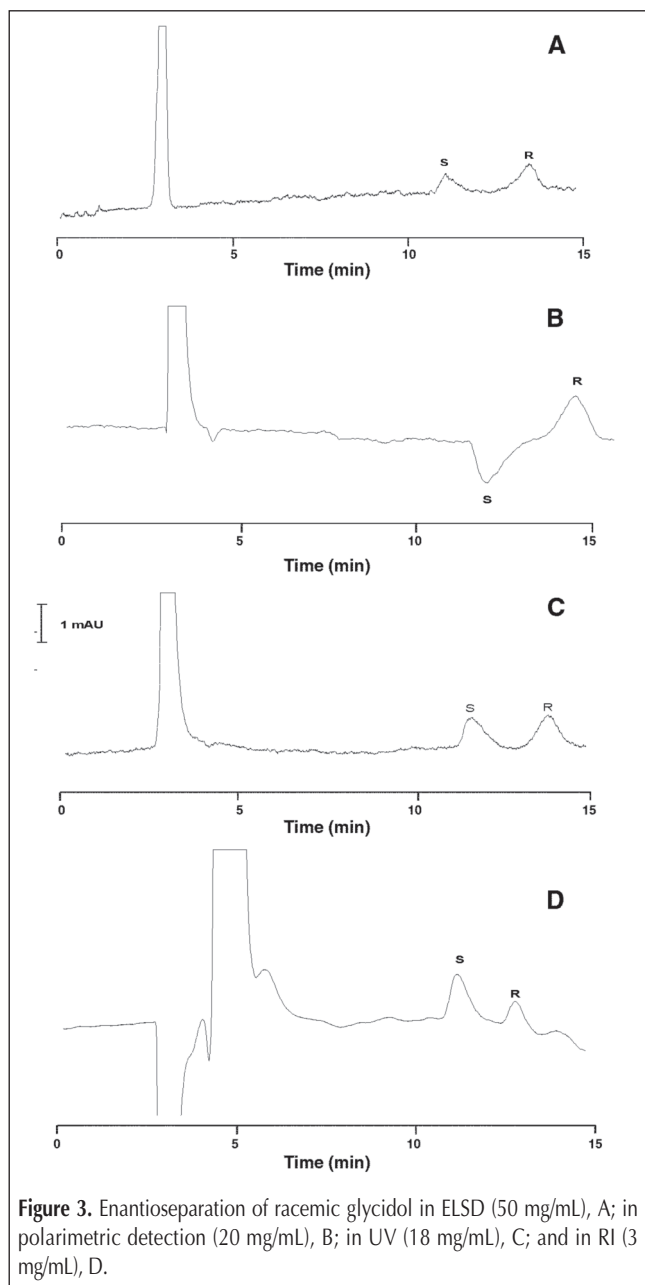
	(*S*)-3-*tert*-butylamino-1,2-propanediol
Exponential model	
Calibration range (µg/mL)	50–1500
Calibration points	6
Equation for which *y* = (*S*)-/(*R*)-form area and *x* = (*S*)-/(*R*)-form concentration	$y = 0.8564\, x^{1.5638}$
Coefficient of determination (r^2)	0.9988
Linear model	
Calibration range (µg/mL)	500–1000
Calibration points	3
Equation for which *y* = log[(*S*)-/(*R*)-form area] and *x* = log[(*S*)-/(*R*)-form concentration]	$y = 1.3723x + 0.1723$
Coefficient of determination (r^2)	0.9995
Second-order polynomial model	
Calibration range (µg/mL)	50–1500
Calibration points	6
Equation for which *y* = log[(*S*)-/(*R*)-form area] and *x* = log[(*S*)-/(*R*)-form concentration)	$y = -0.1349x^2 + 1.7418x - 0.0845$
Coefficient of determination (r^2)	0.9996
Accuracy (n = 6)	
Recovery ± CI (%) at 50 µg/mL	100.5 ± 0.8
Recovery ± CI (%) at 750 µg/mL	101.8 ± 0.9
Recovery ± CI (%) at 1500 µg/mL	98.0 ± 1.7
Repeatability (n = 6, RSD%)	
50 µg/mL	0.7
750 µg/mL	0.9
1500 µg/mL	1.7

background signal was observed because of the mobile phase that was composed of *n*-hexane and ethanol (90:10, v/v). Compared with the observations made in 3-*tert*-butylamino-1,2-propanediol determination, this indicates that the presence of salts in the mobile phase was responsible for a background signal having a detrimental effect on the detection sensitivity.
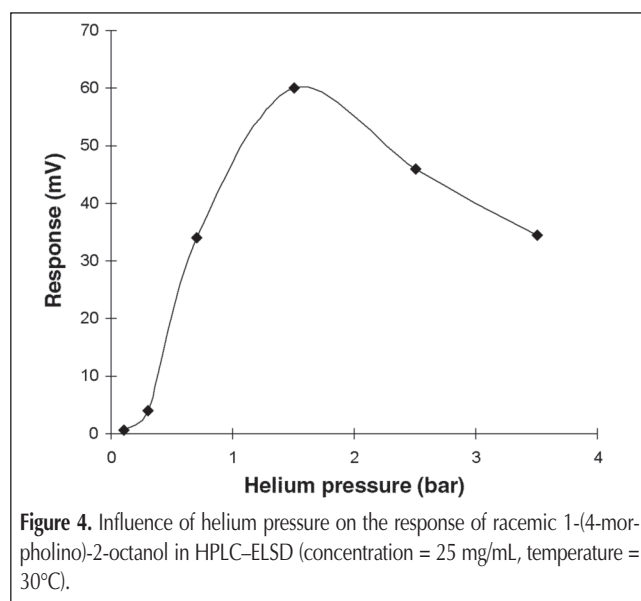
1-(4-Morpholino)-2-octanol

(*R*)- and (*S*)-1-(4-morpholino)-2-octanol were separated according to the method of Nicholson et al. (25). An Rs of 2.1 was obtained.

The detection sensitivity of 1-(4-morpholino)-2-octanol was investigated at 25, 30, 32, 35, and 40°C using helium pressures ranging from 0.1 to 3.5 bar. 1-(4-Morpholino)-2-octanol showed a maximum response at 30°C and 1.3 bar (Figure 4). The LOQs for 1-(4-morpholino)-2-octanol were finally recorded at 1.1 and 2.1 mg/mL for (*R*)- and (*S*)-enantiomers, respectively. As for glycidol,

Figure 4. Influence of helium pressure on the response of racemic 1-(4-morpholino)-2-octanol in HPLC–ELSD (concentration = 25 mg/mL, temperature = 30°C).

Figure 3. Enantioseparation of racemic glycidol in ELSD (50 mg/mL), A; in polarimetric detection (20 mg/mL), B; in UV (18 mg/mL), C; and in RI (3 mg/mL), D.
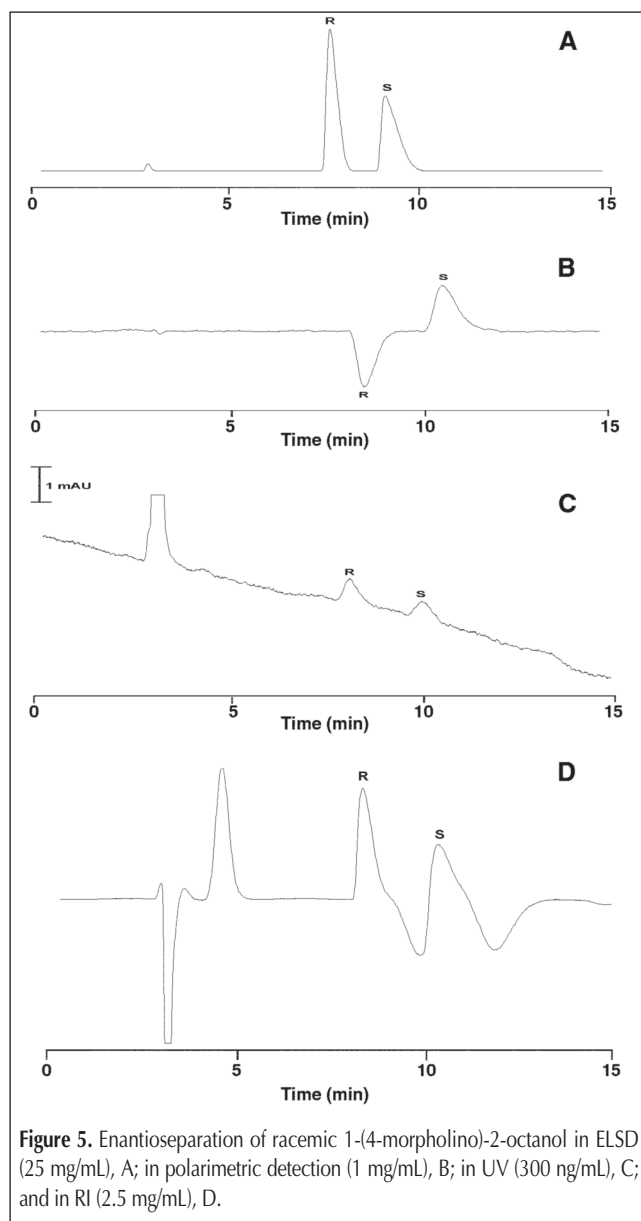
Figure 5. Enantioseparation of racemic 1-(4-morpholino)-2-octanol in ELSD (25 mg/mL), A; in polarimetric detection (1 mg/mL), B; in UV (300 ng/mL), C; and in RI (2.5 mg/mL), D.

no background was generated by the HPLC mobile phase (*n*-hexane/ethanol, 95:5, v/v). The enantioseparation of (*R*,*S*)-1-(4-morpholino)-2-octanol is illustrated in Figure 5A.

The repeatability of the response was also studied and compared with that of 3-*tert*-butylamino-1,2-propanediol by ELSD. The results are presented in Table II. Higher RSD values were observed with (*R*)- and (*S*)-1-(4-morpholino)-2-octanol than with 3-*tert*-butylamino-1,2-propanediol. The volatility of 1-(4-morpholino)-2-octanol is the most likely cause (the analyte evaporates to a large extent in the drift tube; and at 3.3 bar of helium, the nebulization process is much more efficient). However, as in the case of 3-*tert*-butylamino-1,2-propanediol, RSD values of (*R*)-/(*S*)-1-(4-morpholino)-2-octanol area ratios were seven times better than the RSD values for that of one enantiomer alone. This confirms that the vaporization process can vary significantly from run to run and day to day; therefore, an internal standard should be used for quantitation.
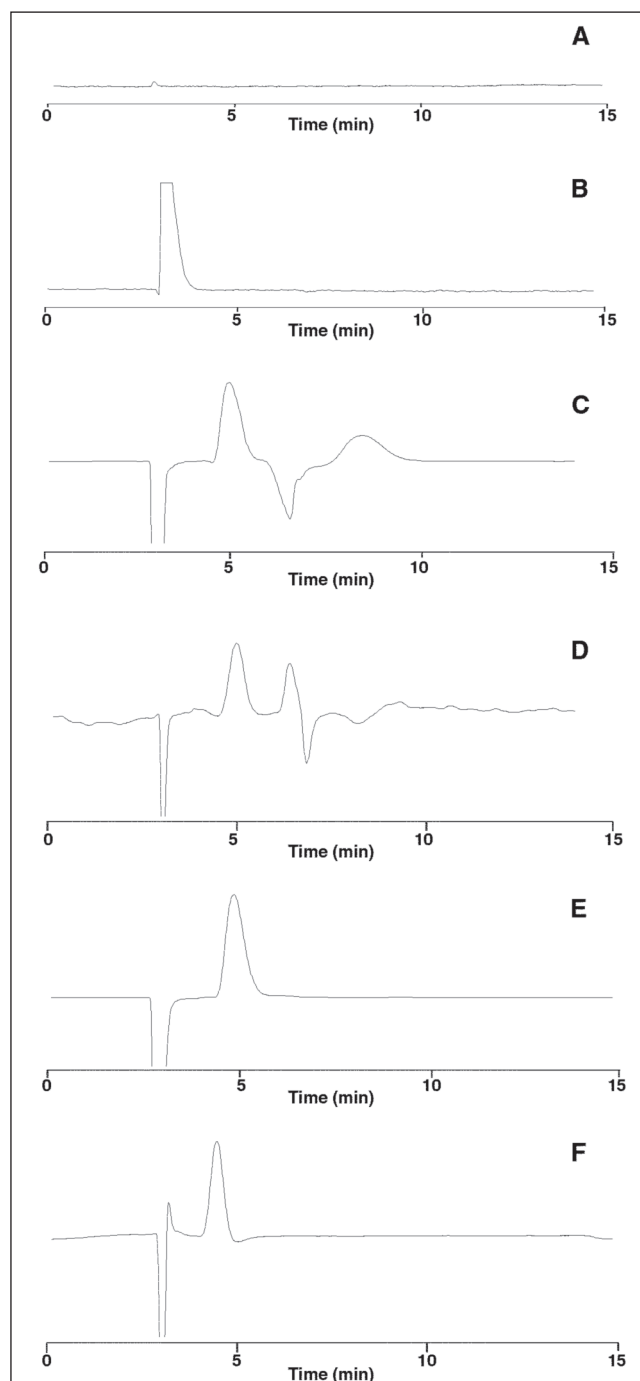
Polarimetric detection
3-tert-*Butylamino-1,2-propanediol*

The LOQs for (*R*)- and (*S*)-3-*tert*-butylamino-1,2-propanediol in polarimetric detection were 0.5 and 0.7 mg/mL, respectively. Figure 6 shows blank chromatograms obtained by the injection of the mobile phase in HPLC–ELSD (Figure 6A) and HPLC–polarimetric detection (Figure 6B). Although polarimetric detection is more selective than ELSD, an increased noise level dramatically decreases the detectability. The enantioseparation of 3-*tert*-butylamino-1,2-propanediol is illustrated in Figure 2B.

In the same way as with ELSD, a one-day validation was performed for (*S*)-3-*tert*-butylamino-1,2-propanediol in the range of 0.75–3.75 mg/mL. Higher concentrations of 3-*tert*-butylamino-1,2-propanediol led to a signal overloading of the detector. The results are presented in Table III. The area of (*S*)-3-*tert*-butylamino-1,2-propanediol was used for calculation without an internal standard. No linear curve could be obtained even after square root or inverse transformation of the data. However, as reported in Table III, a curve corresponding to a second-order polynomial equation was observed (r^2 = 0.9993). This result was similar to that obtained for 3-*tert*-butylamino-1,2-propanediol by

ELSD after logarithmic transformation of the data. A linear model could be applied in the range of 1 to 3.75 mg/mL (r^2 = 0.9994). However, the second-order polynomial model covered the whole concentration range studied.

As shown in Table III, satisfactory results were obtained with

Figure 6. Blank chromatograms obtained by injection of 3-*tert*-butylamino-1,2-propanediol mobile phase in HPLC–ELSD, A; 3-*tert*-butylamino-1,2-propanediol mobile phase in HPLC-polarimetric detection, B; 3-*tert*-butylamino-1,2-propanediol dissolution (with *n*-hexane/ethanol/formic acid/diethylamine, 60:40:0.2:0.2, v/v/v/v being the solvent used) in RI, C; 3-*tert*-butylamino-1,2-propanediol mobile phase in RI, D; glycidol dissolution (with *n*-hexane/ethanol, 60:40, v/v being the solvent used) in RI, E; and 1-(4-morpholino)-2-octanol mobile phase in RI, F.

Table II. Repeatability of the Method Developed for 1-(4-Morpholino)-2-Octanol Determination by HPLC–ELSD* (A) and 3-*tert*-Butylamino-1,2-Propanediol Determination by LC–ELSD   (B)

A	(*R*)-1-(4-morpholino)-2-octanol area	(*S*)-1-(4-morpholino)-2-octanol area	(*R*)-/(*S*)-1-(4-morpholino)-2-octanol area
RSD (%)	12.1	13.8	1.9
B	(*R*)-3-*tert*-butylamino-1,2-propanediol area	(*S*)-3-*tert*-butylamino-1,2-propanediol area	(*R*)-/(*S*)-3-*tert*-butylamino-1,2-propanediol area
RSD (%)	4.2	4.1	0.8

* Racemic 1-(4-morpholino)-2-octanol concentration = 25 mg/mL, *n* = 6, helium pressure = 1.3 bar, and drift tube temperature = 30°C.
† Racemic 3-*tert*-butylamino-1,2-propanediol concentration = 2.0 mg/mL, *n* = 6, helium pressure = 3.3 bar, and drift tube temperature = 45°C.

respect to repeatability. Therefore, no internal standard had to be used for quantitation in contrast with ELSD. Moreover, the (*S*)-/(*R*)-3-*tert*-butylamino-1,2-propanediol area ratio was closer to 1.0 in polarimetric detection than in ELSD. The mean value of the (*S*)-/(*R*)-3-*tert*-butylamino-1,2-propanediol area ratio was 0.94 in polarimetric detection instead of 0.84 in ELSD.

The tested procedure could be considered accurate with a mean recovery of 99.6% and a CI of 1.8%, which is quite similar to ELSD.

Glycidol

LOQs for (*R*)- and (*S*)-glycidol were found to be 9.3 mg/mL. The LOQs are thus two times better than with ELSD. Figure 3B illustrates the enantioseparation of glycidol in polarimetric detection.

1-(4-Morpholino)-2-octanol

LOQs for (*R*)- and (*S*)-1-(4-morpholino)-2-octanol were 0.5 and 0.6 mg/mL, respectively, which is at least two times better than with ELSD. A typical chromatogram of 1-(4-morpholino)-2-octanol is shown in Figure 5B.

UV detection
3-tert-*Butylamino-1,2-propanediol*

The absence of chromophore in 3-*tert*-butylamino-1,2-propanediol and the presence of diethylamine in the HPLC mobile phase made UV detection quite insensitive towards 3-*tert*-butylamino-1,2-propanediol (Figure 2C). The following wavelengths were tested: 210, 220, 230, and 240 nm. As expected, the noise level decreased with increasing wavelength because of the diethylamine as well as the 3-*tert*-butylamino-1,2-propanediol response. An LOQ of 20 mg/mL was observed for both at 210 nm

Table III. Validation Results for (*S*)-3-*tert*-Butylamino-1,2-Propanediol Using HPLC–Polarimetric Detection

	(*S*)-3-*tert*-butylamino-1,2-propanediol
Linear model	
Calibration range (mg/mL)	1–3.75
Calibration points	3
Equation for which y = (*S*)-form area and x = (*S*)-form concentration	$y = 1537.964x + 120.9577$
Coefficient of determination (r^2)	0.9994
Second-order polynomial model	
Calibration range (mg/mL)	0.75–3.75
Calibration points	5
Equation for which y = (*S*)-form area and x = (*S*)-form concentration	$y = 23.441x^2 + 1781.9x - 411.3$
Coefficient of determination (r^2)	0.9993
Accuracy (n = 6)	
Recovery ± CI (%) at 0.75 mg/mL	97.9 ± 2.1
Recovery ± CI (%) at 1.5 mg/mL	100.7 ± 2.3
Recovery ± CI (%) at 3.75 mg/mL	100.1 ± 1.1
Repeatability (n = 6, RSD%)	
0.75 mg/mL	2.1
1.5 mg/mL	2.2
3.75 mg/mL	1.1

enantiomers. Therefore, ELSD seems to be a very useful alternative to UV detection for compounds that do not absorb in UV or when the mobile phase does absorb or during both of these conditions.

Glycidol

Glycidol was detected at 210 nm and the LOQ was 9 mg/mL for both enantiomers. This result was similar to that obtained with polarimetric detection. The UV detection of glycidol enantiomers is illustrated in Figure 3C.

1-(4-Morpholino)-2-octanol

The LOQs for (*R*)- and (*S*)-1-(4-morpholino)-2-octanol were determined at 140 and 180 ng/mL, respectively, at 210 nm (Figure 5C). The detectability for 1-(4-morpholino)-2-octanol is thus far better in UV detection than in ELSD or polarimetric detection. Therefore, if an analyte absorbs sufficiently at low wavelengths and can be analyzed with a UV-transparent mobile phase, then UV detection seems to be more sensitive than ELSD.

RI
3-tert-*Butylamino-1,2-propanediol*

The LOQs for (*R*)- and (*S*)-3-*tert*-butylamino-1,2-propanediol were 1.8 and 2.3 mg/mL, respectively, which is three times better than polarimetric detection but fifty times worse than ELSD. RI detection is more selective than ELSD. However, as can be seen on Figures 6C and 6D, the baseline in a blank chromatogram is strongly influenced by disturbances related to different proportions of *n*-hexane and ethanol used in the sample solution and in the mobile phase. Figure 2D illustrates the injection of (*R*,*S*)-3-*tert*-butylamino-1,2-propanediol. Because of the low solubility of 3-*tert*-butylamino-1,2-propanediol in *n*-hexane, a higher percentage of ethanol had to be used in the sample solution than in the mobile phase. An interference could be observed at the same retention time as the enantiomers. A new optimization of the HPLC conditions would be needed in order to elute 3-*tert*-butylamino-1,2-propanediol in a disturbance-free part of the chromatogram. Considering that the composition of the sample solution was in this case still very close to that of the mobile phase, additional disturbances in the chromatogram could be expected if the injected sample was dissolved in a solvent composition different from that of the mobile phase or, even worse, in a biological sample.

The chromatographic peak of (*S*)-3-*tert*-butylamino-1,2-propanediol could be integrated and quantitated when the profile of a blank chromatogram is taken into account. The validation of the method was again performed in one day at five different concentration levels. As for ELSD and polarimetric detection, no linear curve was observed within the concentration range of 2.5 to 12.5 mg/mL even after square-root or inverse transformation of the data. A second-order polynomial equation was found to fit better with the data ($r^2 = 0.999$) (Table IV). However, a linear curve could be obtained in the range of 3.5 to 7.5 mg/mL ($r^2 = 0.9999$). The second-order polynomial equation was selected for validation.

As in the case of polarimetric detection, the (*S*)-/(*R*)-3-*tert*-butylamino-1,2-propanediol area ratio was approximately 1.0, and no internal standard had to be used for quantitation in contrast with ELSD.

The tested procedure could be considered accurate with a mean recovery of 100.5% and a CI of 0.5%. The less-accurate results observed at the lowest concentration (2.5 mg/mL) could be explained by the high noise level generated by the sample matrix. However, quantitation is limited to the low concentration levels in which peaks are smaller than the baseline jump and can be easily integrated at the top of it. At higher concentrations, bigger peaks include and hide the baseline disturbance, which results in an overestimation of the integration of the peaks.

Glycidol

The LOQs were found to be at 1.5 and 0.9 mg/mL for (*R*)- and (*S*)-glycidol, respectively (Figure 3D). This is between six and ten times better than polarimetric and UV detection. However, the chromatogram of a blank (injection of the solvent used for sample dissolution, Figure 6E) showed less baseline disturbances than in the determination method of 3-*tert*-butylamino-1,2-propanediol.

Table IV. Validation Results for (*S*)-3-*tert*-Butylamino-1,2-Propanediol Using HPLC–RI Detection

	(*S*)-3-*tert*-butylamino-1,2-propanediol
Linear model	
Calibration range (mg/mL)	3.5–7.5
Calibration points	3
Equation for which y = (*S*)-form area and x = (*S*)-form concentration	$y = 169.8938x - 627.7403$
Coefficient of determination (r^2)	0.9999
Second-order polynomial model	
Calibration range (mg/mL)	2.5–12.5
Calibration points	5
Equation for which y = (*S*)-form area and x = (*S*)-form concentration	$y = -1.3024x^2 + 186.23x - 634.3$
Coefficient of determination (r^2)	0.999
Accuracy (n = 6)	
Recovery ± CI (%) at 2.5 mg/mL	103.6 ± 0.3
Recovery ± CI (%) at 5 mg/mL	98.1 ± 0.6
Recovery ± CI (%) at 12.5 mg/mL	99.8 ± 0.5
Repeatability (n = 6, RSD %)	
2.5 mg/mL	0.3
5 mg/mL	0.6
12.5 mg/mL	0.5

Thus, disturbances were probably related to the presence of diethylamine and formic acid in the mobile phase used for 3-*tert*-butylamino-1,2-propanediol analysis.

1-(4-Morpholino)-2-octanol

The LOQs for (*S*)- and (*R*)-1-(4-morpholino)-2-octanol were 0.2 and 0.3 mg/mL, respectively. This is a thousand times less sensitive than UV detection but six times better than ELSD. The noise level was the same for 1-(4-morpholino)-2-octanol and glycidol, probably because the mobile phase compositions were very close. However, two large disturbances were observed at the end of (*S*)- and (*R*)-1-(4-morpholino)-2-octanol chromatographic peaks (Figure 5D). These disturbances appeared as negative peaks and were not observed in the blank (Figure 6F). They seemed to be related again to the use of a higher percentage of ethanol needed for analyte dissolution in the sample compared with that of the mobile phase. These disturbances made the integration of the peaks less precise.

Conclusion

This study presented a comparative evaluation of the detection sensitivity and linearity of the response of ELSD, UV, polarimetric, and RI detection coupled with HPLC for the separation and quantitation of the enantiomers of three chiral aliphatic alcohols (the results are summarized in Table V). LOQs of 26 and 48 µg/mL could be obtained for (*R*)- and (*S*)- 3-*tert*-butylamino-1,2-propanediol in HPLC–ELSD. Compared with ELSD, the other detectors studied showed lower sensitivity for 3-*tert*-butylamino-1,2-propanediol quantitation. Although, it should be noted that the most sensitive detection mode for glycidol was refractometry. However, the strong influence of the sample matrix on the baseline of the chromatogram made quantitation less precise. The LOQs were 0.9 and 1.5 mg/mL for (*S*)- and (*R*)-glycidol, respectively. As can be seen with 1-(4-morpholino)-2-octanol, the analyte exhibited enough UV-absorptive properties using a UV-transparent mobile phase; therefore, the UV detection mode was the most sensitive of all four detection techniques. LOQs of 140 and 180 ng/mL were obtained for (*R*)- and (*S*)-1(-4-morpholino)-2-octanol, respectively. This was between 1000 and 7000 times better than with the other detection modes. The results of this comparison study clearly indicate that the potential of ELSD, UV, polarimetric,

Table V. Comparison Between the Detection Sensitivity of the Four Detectors*

	ELSD		Polarimetry		UV (210 nm)		RI	
	LOQ (mg/mL)	Noise (mV)	LOQ (mg/mL)	Noise (mV)	LOQ (mg/mL)	Noise (mV)	LOQ (mg/mL)	Noise (mV)
(*R*)-3-*tert*-butylamino-1,2-propanediol	**2.6×10^{-2}**	0.07	0.5	5	20.0	115	1.8	1.5
(*S*)-3-*tert*-butylamino-1,2-propanediol	**4.8×10^{-2}**	0.07	0.7	5	20.0	115	2.3	1.5
(*R*)-glycidol	19.5	0.21	9.3	6	9.0	0.12	**1.5**	0.3
(*S*)-glycidol	19.5	0.21	9.3	6	9.0	0.12	**0.9**	0.3
(*R*)-1-(4-morpholino)-2-octanol	1.1	0.07	0.5	5	**1.4×10^{-4}**	0.12	0.2	0.3
(*S*)-1-(4-morpholino)-2-octanol	2.1	0.07	0.6	5	**1.8×10^{-4}**	0.12	0.3	0.3

* Best results are in bold type.

and RI detection for the enantiomeric purity testing of compounds with poor or no chromophoric properties depends very much upon the physical and chemical properties of the compound examined and the composition of the HPLC mobile phase.

Acknowledgments

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