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Journal of Chromatography A, 890 (2000) 239–249

JOURNAL OF CHROMATOGRAPHY A

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Determination of the enantiomers of 3-*tert*.-butylamino-1,2-propanediol by high-performance liquid chromatography coupled to evaporative light scattering detection

B. Toussaint[a,*], A.L.L. Duchateau[b], Sj. van der Wal[b], A. Albert[c], Ph. Hubert[a], J. Crommen[a]

[a]*Department of Analytical Pharmaceutical Chemistry, Institute of Pharmacy, University of Liège, CHU, B 36, B-4000 Liège, Belgium*
[b]*DSM Research, P.O. Box 18, 6160 MD Geleen, The Netherlands*
[c]*Department of Biostatistics, University of Liège, CHU, B 23, B-4000 Liège, Belgium*

Received 28 January 2000; received in revised form 10 May 2000; accepted 23 May 2000

Abstract

A method for the separation and quantitation of the enantiomers of 3-*tert*.-butylamino-1,2-propanediol by high-performance liquid chromatography and evaporative light scattering detection has been developed. Separation of the enantiomers was performed in normal-phase liquid chromatography on a Chiralpak AS chiral stationary phase. The influence of the gas nature, gas pressure and temperature of the drift tube of the evaporative light scattering detector on the detection sensitivity was investigated. The method was validated in terms of linearity, limit of quantitation, accuracy and precision. The enantiomeric excess of (*S*)-3-*tert*.-butylamino-1,2-propanediol, used for the industrial synthesis of (*S*)-timolol, was measured from 0 to 94%.

Keywords: Enantiomer separation; Evaporative light scattering detection; 3-*tert*.-Butylamino-1,2-propanediol

1. Introduction

A major part of the compounds that are used in the pharmaceutical industry are chiral [1]. The enantiomeric purity of these products is most often determined by high-performance liquid chromatography (HPLC). However, for some important types of chiral compounds, e.g., non-aromatic amino alcohols, few standard analytical methods are available for enantioseparation and detection. Ultraviolet absorption detection (UV) has been used for many years as the most popular form of detection in liquid chromatographic separations [2]. However, analytes containing weak or no chromophores can remain undetected and need to be derivatized before or after separation [3–5]. Unlike UV and fluorescence detection, evaporative light scattering detection (ELSD) represents a universal detection mode suitable for non-absorbing analytes [3,6–11]. Three main processes occur sequentially in ELSD: (1) nebulization of the chromatographic eluent with an inert gas; (2) evaporation of the mobile phase in a heated zone (the 'drift tube'); and (3) detection of

*Corresponding author. Tel.: +32-4-3664-354; fax: +32-4-3664-347.
E-mail address: b.toussaint@ulg.ac.be (B. Toussaint).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00609-9

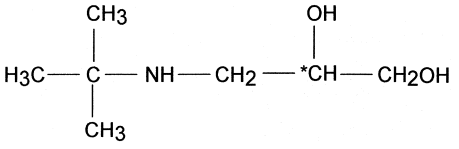

Fig. 1. Chemical structure of 3-*tert*.-butylamino-1,2-propanediol.

light scattered by the remaining particles. As the response does not depend on the solute optical properties, any compound appreciably less volatile than the mobile phase can be detected, regardless of its functional groups. Moreover, mobile phases can be selected based on substance solubility and separation characteristics without regard of UV transparency.

This paper describes a HPLC method for the separation and quantitation of the enantiomers of 3-*tert*.-butylamino-1,2-propanediol. The (*S*)-enantiomer of this amino alcohol is used as an intermediate in the industrial synthesis of β-blockers like (*S*)-timolol. 3-*tert*.-butylamino-1,2-propanediol shows low absorption properties in the UV region. Its chemical structure is illustrated in Fig. 1. An HPLC-ELSD method was developed and validated with respect to linearity, detectability, accuracy and precision. The influence of the gas nature, gas pressure and temperature of the drift tube on 3-*tert*.-butylamino-1,2-propanediol response using the ELSD detector was studied. The applicability of this method to the determination of the enantiomeric excess of (*S*)-3-*tert*.-butylamino-1,2-propanediol was then investigated.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical grade. Ethanol, *n*-hexane and formic acid 98–100% were obtained from Merck (Darmstadt, Germany). Diethylamine 99% was from Fluka. (*R,S*)-3-*tert*.-butylamino-1,2-propanediol and (*S*)-3-*tert*.-butylamino-1,2-propanediol, were kindly offered by DSM Fine Chemicals (Venlo, The Netherlands).

Sample solutions were prepared by dissolving (*R,S*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol in the mobile phase. The limits of determination (LOD) and quantitation (LOQ) of both enantiomer were determined by use of racemic (*R,S*)-3-*tert*.-butylamino-1,2-propanediol. The calibration curve of the (*S*)-enantiomer was obtained using samples containing 50 μg/ml of racemic 3-*tert*.-butylamino-1,2-propanediol and increasing amounts of the (*S*)-enantiomer in order to reach 200, 500, 750, 1000 and 1500 μg/ml in (*S*)-3-*tert*.-butylamino-1,2-propanediol. In the same manner, the calibration curve of the (*R*)-enantiomer was obtained employing samples containing 45, 200, 500, 750, 1000 and 1500 μg/ml of racemic 3-*tert*.-butylamino-1,2-propanediol and increasing amounts of the (*S*)-enantiomer in order to keep the (*S*)-enantiomer concentration constant at 1500 μg/ml in all samples.

2.2. Instrumentation and methods

The HPLC system consisted of a model HP-1100 liquid chromatograph equipped with a quaternary pump from Hewlett-Packard (Palo Alto, CA, USA). The autosampler was a Midas system equipped with a 25-μl injection loop from Spark Holland (Emmen, The Netherlands). Quantification was performed with a LabSystems Xchrom data system (Manchester, UK). The HPLC separations were performed at 25°C. The enantioseparation of 3-*tert*.-butylamino-1,2-propanediol was achieved using a Chiralpak AS column (10 μm, 250 mm×4.6 mm I.D.). A Chiralcel OD-H column (5 μm, 250 mm×4.6 mm i.d) was also tested. All columns were from Daicel Chemical Industries (Tokyo, Japan). The mobile phase consisted of a mixture of *n*-hexane, ethanol, formic acid and diethylamine (90:10:0.2:0.2, v/v/v/v). The mobile phase was degassed for 10 min in an ultrasonic bath before use. The flow-rate was 1.0 ml/min.

The evaporative light scattering detector was a Sedex 55 model from Sedere (Alfortville, France). Helium was used as evaporation gas. The gas pressure and the drift tube temperature could be finely tuned. The gain was set at 5. The syphon of the waste tube, used for the elimination of liquid

excess, was filled with water purified with a Milli-Q system (Millipore, Bedford, MA, USA).

3. Results and discussion

3.1. Optimization of 3-tert.-butylamino-1,2-propanediol enantioseparation

Two different chiral stationary phases were tested: Chiralpak AS and Chiralcel OD-H which consist of amylose tris((*S*)-methylbenzylcarbamate) and cellulose tris(3,5-dimethylphenyl-carbamate), respectively, coated on silica gel. No enantioseparation of 3-*tert*.-butylamino-1,2-propanediol could be obtained on the Chiralcel OD-H column. However, a baseline separation was obtained on the Chiralpak AS column. This confirms the usefulness of the Chiralpak AS column for the enantioseparation of aliphatic and low-molecular weight samples compatible with normal-phase chromatography [12].

The composition of the mobile phase was also investigated. The influence of the nature of the alcohol (ethanol, 2-isopropanol and mixtures of both), the percentage of alcohol and the addition of a modifier such as formic acid and diethylamine were studied. The best separation of 3-*tert*.-butylamino-1,2-propanediol enantiomers was obtained with a mixture of *n*-hexane, ethanol, formic acid and diethylamine (90:10:0.2:0.2; v/v/v/v). The retention factors (k') of (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol were 1.75 and 2.38, respectively, and the enantioresolution (R_s) was 2.65. Diethylamine is often used as a modifier for basic samples in order to ensure their elution from the column in normal-phase chromatography [12]. In this case, a significant improvement of the peak shape could be observed after addition of 0.2% diethylamine to the mobile phase. On the other hand, no enantioseparation could be obtained with a mixture of *n*-hexane, ethanol and diethylamine without formic acid. Formic acid probably promotes the ionization of the secondary amine of 3-*tert*.-butylamino-1,2-propanediol and might form an ion pair, improving the interaction of the enantiomers with the stationary phase and increasing retention. When a higher percentage of that modifier was used in the mobile phase, e.g., 0.3% formic acid, no elution of 3-*tert*.-butylamino-1,2-propanediol could be observed within 40 min.

3.2. Optimization of the ELSD

The influence of the gas nature (air and helium), the gas pressure and the drift tube temperature on 3-*tert*.-butylamino-1,2-propanediol response were studied.

As is well known, the residence time in the drift tube must be large enough and the kinetics of heat transfer in this tube between the gas and the nebulized sample droplets must be fast enough to ensure complete vaporization of the solvents [13–15]. Thanks to its small molecular diameter, helium gives better heat transfer and better detection sensitivity than air and was selected as the most suitable carrier gas [16].

The gas pressure and the temperature of the drift tube are also of the utmost importance to ensure sufficient vaporization of the HPLC mobile phase while the analytes remain in particle form. Therefore, the boiling point of the compound has to be higher than the one of the mobile phase. The boiling point of 3-*tert*.-butylamino-1,2-propanediol is 265°C (at 1 atm).

The detection sensitivity of 3-*tert*.-butylamino-1,2-propanediol was investigated using helium pressures from 0.1 to 4.0 bar and temperatures from 30 to 50°C. The vaporization process could be evaluated by observing the background, i.e., the light scattered when only mobile phase was passing the detector. At 30°C the background increases with the gas pressure indicating that the mobile phase undergoes insufficient vaporization in the drift tube and solvent droplets are formed. As the gas pressure increases, more efficient nebulization takes place and more droplets reach the detector, scattering more and more light. At 35, 40 and 45°C the background shows an increase from 0.1 to 1.5 bar before decreasing from 1.5 to 4.0 bar. Indeed, the background is around zero at very low gas pressure as the liquid mobile phase goes directly to waste. As the gas pressure increases, more mobile phase droplets enter the drift tube generating background. Then around 1.5 bar, the gas pressure allows better nebulization creating smaller droplets and causing at those temperatures a partial

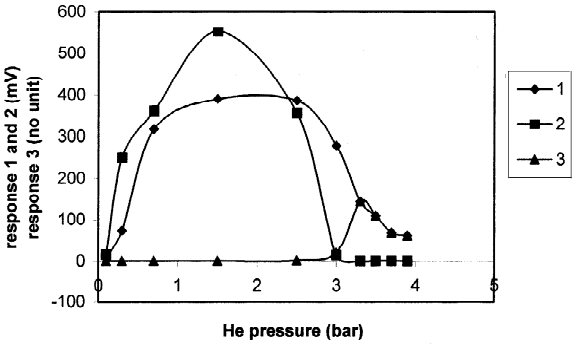

Fig. 2. Influence of He pressure on: (1) the response of (*R*)-3-*tert*.-butylamino-1,2-propanediol in HPLC-ELSD (mV); (2) the background intensity (mV); (3) the ratio of (*R*)-3-*tert*.-butylamino-1,2-propanediol with the background. Conditions: racemic 3-*tert*.-butylamino-1,2-propanediol concentration, 2 mg/ml; temperature, 45°C.

evaporation of the mobile phase. Complete evaporation is finally observed around 3.5 bar. At 50°C, the mobile phase is entirely evaporated even at low gas pressure. However, gas pressure and temperature have to be set as low as possible in order to prevent sample loss by evaporation. The use of a mobile phase without diethylamine at 45°C and 3.5 bar of He pressure shows a lower background than a mobile phase containing both formic acid and diethylamine indicating that possibly non-volatile salt formation could be responsible for the main part of the remaining background. This confirm what was reported in literature [17].

The limit of quantitation (LOQ) of 3-*tert*.-butylamino-1,2-propanediol in ELSD is of course related to the background level. The influence of the gas pressure on 3-*tert*.-butylamino-1,2-propanediol response was investigated at 30, 35, 40, 45 and 50°C. A maximum response was observed around 1.5 bar at any temperature. A representative graph for 45°C is shown in Fig. 2. Taking the background into account, the best LOQ for 3-*tert*.-butylamino-1,2-propanediol was obtained at 45°C and 3.3 bar (Fig. 3).

Knowing the strong influence of the gas pressure on the detection sensitivity, another parameter to be aware of is the reproducibility of the gas–liquid equilibrium in the drift tube and the stability of the liquid level in the syphon of the waste tube. The waste tube is directly connected to the drift tube and collects bigger eluent droplets that do not evaporate easily. The syphon has to be filled, usually with mobile phase, before any analysis as the liquid phase in the syphon is in equilibrium with the gas phase in the drift tube. Approximately 1.5 h is needed in order to reach a stable equilibrium state. A slight decrease in peak areas could be observed during the first injections of 3-*tert*.-butylamino-1,2-propanediol enantiomers, leading to an apparent relative standard deviation (RSD) of 18.6% for six first injections

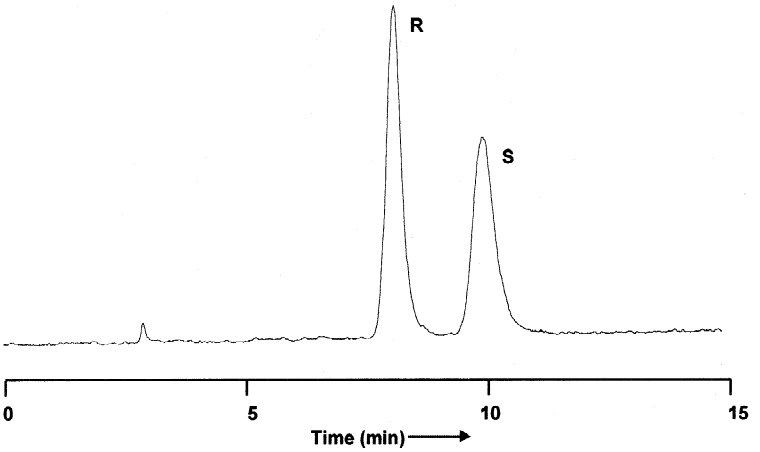

Fig. 3. Enantioseparation of racemic 3-*tert*.-butylamino-1,2-propanediol (200 μg/ml) by HPLC-ELSD at 45°C with a He pressure of 3.3 bar.

instead of 6.3% after 1.5 h. As both enantiomers undergo the same changes in sensitivity, an internal standard is strongly recommended in order to perform quantitative analysis. In this case, one enantiomer could be selected as an internal standard for the quantitation of the other one. An RSD of 0.9% was obtained using (*S*)-/(*R*)-3-*tert*.-butylamino-1,2-propanediol area ratio.

In addition, any variation of the liquid level of the waste tube leads to a change in the gas pressure within the drift tube. Unfortunately, this level tends to decrease by evaporation even at 30°C. In order to keep this level constant during analyses, the syphon of the waste tube was filled with a less volatile solvent, e.g., water. In this way, no change in the liquid level in the waste tube was observed for at least 8 h improving the reproducibility of the experiments (Table 1). The RSD observed for (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol with water in the waste tube was 2% lower than with mobile phase.

In order to study the possible influence of the mobile phase composition on the repeatability of the response, RSDs were determined for 3-*tert*.-butylamino-1,2-propanediol using a mobile phase without diethylamine so that no salts could be formed (R_s=1.7). Similar RSDs were obtained for 3-*tert*.-butylamino-1,2-propanediol with or without diethylamine in the mobile phase indicating that salt formation does not decrease the repeatability of the response (Table 1).

In conclusion, parameters related to the equilibration and the conditioning of the ELSD are mainly responsible for day to day response variations and are quite difficult to control accurately. Very small variations of the parameters described above as well as the position or the dimensions of the drift tube can dramatically influence quantitative results [13–15]. Therefore the use of an internal standard in quantitative ELSD studies is definitely of the utmost importance.

3.3. Method validation

The method for the enantioseparation and the quantitation of 3-*tert*.-butylamino-1,2-propanediol enantiomers by HPLC-ELSD was validated over a 3-day period (k=3) for the (*S*)-form. A 1-day validation (k=1) was also performed for the (*R*)-enantiomer.

3.3.1. Validation of the method developed for the determination of (S)-3-tert.-butylamino-1,2-propanediol

3.3.1.1. Selectivity

Upon inspection of the chromatogram of the mobile phase used for 3-*tert*.-butylamino-1,2-propanediol dissolution no interference was observed at the retention times of (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol.

3.3.1.2. Linearity

The calibration curve was constructed at six concentration levels of (*S*)-3-*tert*.-butylamino-1,2-propanediol in the range 50–1500 μg/ml. Three independent determinations were performed at each concentration (n=3) except the lowest, the highest

Table 1
Repeatability of the method developed for 3-*tert*.-butylamino-1,2-propanediol determination by HPLC-ELSD with and without diethylamine in the HPLC mobile phase and using mobile phase or water in the syphon of the waste tube (concentration of racemic 3-*tert*.-butylamino-1,2-propanediol=2.0 mg/ml, n=6)[a]

Mobile phase	In the syphon	RSD (%) of (*R*)-form area	RSD (%) of (*S*)-form area	RSD (%) of (*R*)/(*S*)-form area
n-Hexane:ethanol:formic acid:diethylamine (90:10:0.2:0.2, v/v/v/v)	Mobile phase	6.3	6.2	0.9
	Water	4.2	4.1	0.8
n-Hexane:ethanol: formic acid (90:10:0.2, v/v/v)	Water	3.7	3.2	1.5

[a] He pressure and drift tube temperature were 3.3 bar and 45°C, respectively.

and the medium points of the scale, where six independent determinations were achieved ($n=6$) improving precision for extreme values. In order to avoid variations due to injection or sample vaporization in ELSD, the (*R*)-enantiomer at the concentration of 50 μg/ml was considered as an internal standard for the quantitation of the (*S*)-form. Taking into account the solubility and the limit of quantitation of the enantiomers, the six concentration levels of the calibration curve were selected in order to cover a range of (*S*)-/(*R*)-3-*tert*.-butylamino-1,2-propanediol ratios from 50/50 to 96.8/3.2. This corresponds to an enantiomeric excess range of 0–94% for (*S*)-3-*tert*.-butylamino-1,2-propanediol. The (*S*)-/(*R*)-3-*tert*.-butylamino-1,2-propanediol area and concentration ratio were used for calculation. An exponential curve was obtained by plotting the peaks area ratio (y) versus (*S*)-/(*R*)-3-*tert*.-butylamino-1,2-propanediol concentration in μg/ml (x) (Table 2). The results confirm that unlike traditional HPLC detectors, such as UV or RI, the ELSD is often non-linear [8,13,14,16]. The logarithmic transformation of the data gave a curve corresponding to a two-order polynomial equation with a coefficient of determination (r^2) of 0.9996. However, this does not exclude the ability to use this detector to obtain quantitative results. Two alternatives can be proposed. The first of them is based on the fact that all non-linear curves have a region/range of linear response. Indeed, data in the range 500–1000 μg/ml were fitted to the logarithmic regression: $\log A = b \log m + \log a$, where A is the ratio of (*S*)-/(*R*)-3-

Table 2
Validation results for (*S*)-3-*tert*.-butylamino-1,2-propanediol

	(*S*)-3-*tert*.-Butylamino-1,2-propanediol
Exponential model:	
Calibration range (μg/ml)	50–1500
Calibration points	6
Equation, where y=(*S*)-/(*R*)-form area and x=(*S*)-/(*R*)-form concentration	$y=0.8564x^{1.5638}$
Coefficient of determination (r^2)	0.9988
Linear model:	
Calibration range (μg/ml)	500–1000
Calibration points	3
Equation, where 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, where 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 ($k=1$, $n=6$):	
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 ($k=3$, $n=6$, RSD%):	
50 μg/ml	0.6
750 μg/ml	2.0
1500 μg/ml	4.3
Intermediate precision ($k=3$, $n=18$, RSD%):	
50 μg/ml	5.5
750 μg/ml	5.5
1500 μg/ml	5.6

tert.-butylamino-1,2-propanediol area corresponding to the intensity of scattered light, *m* is the mass of the analyte relative to the internal standard, *a* and *b* are ELSD coefficients that depend on the nebulization and evaporation conditions, the chromatographic system and the concentration as well as some properties of the solute [7,8]. The regression line was obtained by using the least-squares method according to the hypothesis of homoscedasticity [18,19]. A coefficient of determination (r^2) of 0.9995 was obtained (Table 2) and the linearity could be confirmed by an analysis of the variance (ANOVA) [18,19]. If the calibration standards and samples are within this range the linear equation can be used for quantitation. Data within that range can be easily generated by sample dilutions or loop volume changes. The linear equation confirms the theoretical observation that *b* values are usually between 0.66 and 2 [20]. On the other hand, the straight line does not pass through the origin as the intercept corresponds to the racemic mixture of 3-*tert*.-butylamino-1,2-propanediol:

$$\log(A_{(S)\text{-pamol}}/A_{(R)\text{-pamol}}) = b\log(m_{(S)\text{-pamol}}/m_{(R)\text{-pamol}}) + a$$

for the racemic mixture:

$$\log(A_{(S)\text{-pamol}}/A_{(R)\text{-pamol}}) = b\ log\ 1 + a$$

that means:

$$log(A_{(S)\text{-pamol}}/A_{(R)\text{-pamol}}) = a$$

This indicates that (*S*)-, and (*R*)-3-*tert*.-butylamino-1,2-propanediol areas are significantly different at equal concentration. Indeed, in the experimental conditions, (*S*)-/(*R*)-3-*tert*.-butylamino-1,2-propanediol area mean value was 0.84. The reason of the difference between (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol response in ELSD is unknown so far.

The second alternative for quantitation is to solve the two-order equation as follows:

$$y = ax^2 + bx + c$$

where y = log((*S*)-/(*R*)-enantiomer area) = observed value and x = log((*S*)-/(*R*)-enantiomer concentration) = unknown value.

The equation becomes:

$$0 = ax^2 + bx + (c - y)$$

and the solution is the positive value of:

$$x_{1,2} = \frac{-b \pm \sqrt{b^2 - 4a(c-y)}}{2a}$$

Using this model, the method could be validated in the whole range of concentration from 50 to 1500 μg/ml. Therefore, no dilution of the samples was needed before analysis and the enantiomeric excess of (*S*)-3-*tert*.-butylamino-1,2-propanediol could be determined in the range 0–94%, instead of 82–90% when using the restricted linear regression. Such a large range is particularly interesting for the determination of the enantiomeric purity of the compound as it is being used as an intermediate in the industrial synthesis of (*S*)-timolol. Indeed, a method for the determination of low (*R*)-enantiomer concentrations in the presence of high (*S*)-enantiomer amounts has to be used in that case and the enantiomeric excess determination of the (*S*)-form is mostly needed in the upper values (>90%). Therefore, the second-order polynomial equation was selected as the best suited for that application and was used for precision and accuracy calculations.

3.3.1.3. Limits of detection and quantitation

The limits of detection (LOD) and quantitation (LOQ) were measured as the concentration of substance giving a signal-to-noise ratio of 3 and 10, respectively. The noise was a peak hyphen to hyphen peak noise. For the (*S*)-3-*tert*.-butylamino-1,2-propanediol enantiomer, the LOD was found to be 15 μg/ml, which corresponds to an amount injected of 0.7 μg. The LOQ was 48 μg/ml, which corresponds to the injection of 1.2 μg of (*S*)-3-*tert*.-butylamino-1,2-propanediol.

3.3.1.4. Precision

Method precision was determined by measuring repeatability and intermediate precision (between-day precision) for the amount of (*S*)-3-*tert*.-butylamino-1,2-propanediol found using the polynomial model. The study was carried out over 3 days (k = 3) at three concentration levels. The RSD values were estimated from the repeatability and the intermediate precision variances of the results, respective-

ly [19,21]. As can be seen in Table 2, acceptable results with respect to precision were obtained.

3.3.1.5. Accuracy

Method accuracy was determined for (*S*)-3-*tert*.-butylamino-1,2-propanediol at three concentration levels ($n=6$) using the polynomial model. Recoveries and confidence intervals (CIs) were calculated:

Recovery = (concentration found/
concentration applied in samples) × 100

$$CI = \frac{t \cdot s}{\sqrt{n}}$$

where t is Student's t-test (0.95; $n-1$); s is standard deviation, and n is number of injections.

As can be seen in Table 2, 100% recovery was not always included in the confidence interval. This was due to the relatively small CIs obtained from particularly low standard deviation values. However, the recoveries were all included in the interval 98–102%. The use of this interval can be found in the European Pharmacopea for drug purity control [22]. The same approach is proposed for accuracy determination by the Validation Guidelines [23,24]. Therefore, the second order polynomial model was accepted and the method was accurate. The procedure was also tested over 3 days and could be considered accurate with a mean recovery of 99.7% and a confidence interval of 1.8% ($k=3$, $n=6$).

3.3.2. Validation of the method developed for the determination of (R)-3-tert.-butylamino-1,2-propanediol

3.3.2.1. Linearity of (R)-3-tert.-butylamino-1,2-propanediol response

The same statistical approach as that described for (*S*)-3-*tert*.-butylamino-1,2-propanediol determination was used. The calibration curve was constructed at six concentration levels in the range 45–1500 μg/ml of (*R*)-3-*tert*.-butylamino-1,2-propanediol. The (*S*)-enantiomer at the concentration of 1500 μg/ml was considered as an internal standard for the quantitation of the (*R*)-form. The six concentration levels of the calibration curve were selected in order to cover a range of (*R*)-/(*S*)-3-*tert*.-butylamino-1,2-propanediol ratios from 50/50 to 2.99/97.1. This corresponds to an enantiomeric excess range of 0–94% for (*S*)-3-*tert*.-butylamino-1,2-propanediol. The (*R*)-/(*S*)-3-*tert*.-butylamino-1,2-propanediol area and concentration ratio were used for calculation. The same type of calibration curve was observed as for (*S*)-3-*tert*.-butylamino-1,2-propanediol (Table 3). The logarithmic transformation of the data gave a curve corresponding to a two-order polynomial equation with a coefficient of determination (r^2) of 0.9994. As for (*S*)-3-*tert*.-butylamino-1,2-propanediol, a linear curve ($r^2=0.9999$) could be obtained with data in the range 45–500 μg/ml. However, the polynomial model was selected in order to be able to analyse samples in the whole range of concentrations.

3.3.2.2. Limits of detection and quantitation

The LOD and LOQ of (*R*)-3-*tert*.-butylamino-1,2-propanediol were found to be 8 and 26 μg/ml, respectively, which correspond to an amount injected of 0.2 and 0.7 μg, respectively.

3.3.2.3. Precision

Repeatability was studied for (*R*)-3-*tert*.-butylamino-1,2-propanediol over 1 day at three concentration levels ($k=1$, $n=6$) using the polynomial model. Satisfactory RSDs values are reported in Table 3.

3.3.2.4. Accuracy

The accuracy of the method was estimated in the polynomial model at three concentration levels ($n=6$). The tested procedure could be considered accurate with a mean recovery of 99.8% and a CI of 1.2%.

3.3.3. Enantiomeric excess determination

Enantiomeric excess (e.e.) determination is of the utmost importance in the pharmaceutical industry in order to control the enantiomeric purity of chiral compounds used directly or as intermediates for the synthesis of therapeutic drugs. As (*S*)-3-*tert*.-butylamino-1,2-propanediol has to be used for the synthesis of (*S*)-timolol, the enantiomeric excess of (*S*)-3-*tert*.-butylamino-1,2-propanediol was determined in standard solutions. The graph of the e.e. found (%) versus the e.e. applied in samples (%) is illustrated in Fig. 4. The second-order polynomial

Table 3
Validation results for (*R*)-3-*tert*.-butylamino-1,2-propanediol

	(*R*)-3-*tert*.-Butylamino-1,2-propanediol
Exponential model:	
Calibration range (μg/ml)	45–1500
Calibration points	6
Equation, where $y=(S)$-/(*R*)-form area and $x=(S)$-/(*R*)-form concentration	$y=0.9728x^{1.4613}$
Coefficient of determination (r^2)	0.9975
Linear model:	
Calibration range (μg/ml)	45–500
Calibration points	3
Equation, where $y=\log((S)$-/(*R*)-form area) and $x=\log((S)$-/(*R*)-form concentration)	$y=1.5712x+0.1264$
Coefficient of determination (r^2)	0.9999
Second-order polynomial model:	
Calibration range (μg/ml)	45–1500
Calibration points	6
Equation, where $y=\log((S)$-/(*R*)-form area) and $x=\log((S)$-/(*R*)-form concentration)	$y=-0.1731x^2+1.2023x-0.0533$
Coefficient of determination (r^2)	0.9994
Accuracy ($k=1$, $n=6$):	
Recovery±CI (%) at 45 μg/ml	99.9±2.0
Recovery±CI (%) at 750 μg/ml	97.7±1.0
Recovery±CI (%) at 1500 μg/ml	101.7±0.6
Repeatability ($k=1$, $n=6$, RSD%):	
45 μg/ml	1.9
750 μg/ml	0.6
1500 μg/ml	0.6

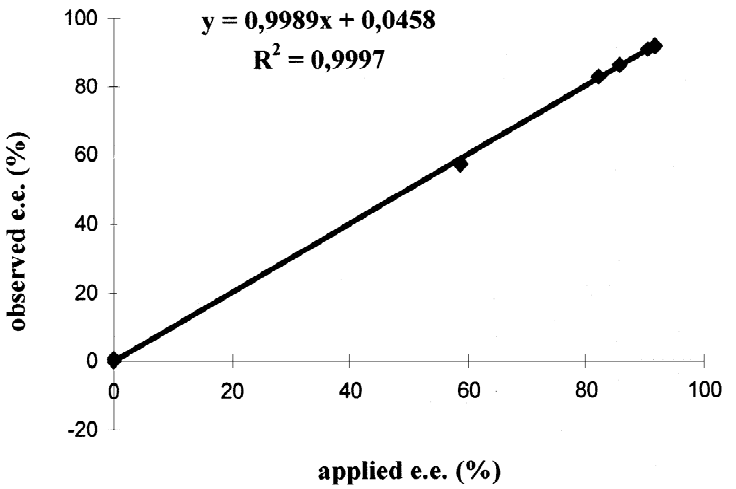

Fig. 4. Enantiomeric excess determination of 3-*tert*.-butylamino-1,2-propanediol by HPLC-ELSD in the range of concentration 50–1500 μg/ml (polynomial model).

equation determined for (*S*)-3-*tert*.-butylamino-1,2-propanediol in the concentration range 50–1500 μg/ml after logarithmic transformation of the data (r^2 = 0.9996) was used for the e.e. calculation. The enantiomeric excess range was 0–94% and is well suited for the determination of low (*R*)-enantiomer concentrations in the presence of high (*S*)-enantiomer concentrations. The slope of the curve was close to 1 indicating a good correlation between observed and applied e.e. The upper limit of 94% is caused by the limited LOQ of the internal standard (26 μg/ml, 0.7 μg injected) and the limited solubility of (*R,S*)-3-*tert*.-butylamino-1,2-propanediol in the mobile phase. However, in many cases of enantiomeric purity control, the enantiomeric excess has to be determined for a sample in the range 0–99%. For example, a sample with an e.e. of 99% and containing (*R*)-3-*tert*.-butylamino-1,2-propanediol as an internal standard quantifiable at the concentration of 30 μg/ml will contain (*S*)-3-*tert*.-butylamino-1,2-propanediol at 5.970 mg/ml. However, the solubility of (*S*)-3-*tert*.-butylamino-1,2-propanediol in the mobile phase is limited to 3 mg/ml. Such a sample could be analysed after dissolution in a solvent containing a higher percentage of ethanol than in the mobile phase, e.g., hexane, ethanol, formic acid and diethylamine in the proportions 60/40/0.2/0.2. However, this could be responsible for peak broadening and precipitation of the sample in the mobile phase. Another alternative in order to determine an e.e. of 99% in such a sample, could be a 4-fold dilution of the sample in the mobile phase. By this way, (*S*)-3-*tert*.-butylamino-1,2-propanediol would be easily dissolved at 1.493 mg/ml. However the (*R*)-form, used as an internal standard, would be 4-fold diluted as well and its concentration would become lower than the LOQ. To overcome this problem, the amount of internal standard injected could be 4-fold increased by increasing the injection volume in HPLC up to 100 μl instead of 25 μl. By that way, (*R*)-3-*tert*.-butylamino-1,2-propanediol could be quantifiable with 0.75 μg injected. Owing to the enantioresolution obtained in the method, a peak broadening of factor 2 is acceptable and an injection volume up to 200 μl could be used without detrimental effect on the enantioseparation. That means that the analysis of a sample at 24 mg/ml of (*S*)-3-*tert*.-butylamino-1,2-propanediol could be performed with this method: first, dilution of the sample down to 3 mg/ml with the mobile phase, second, injection of 200 μl of the diluted sample in HPLC. In conclusion, enantiomeric excesses from 0 to 99.8% for samples containing minimum 26 μg/ml of (*R*)-3-*tert*.-butylamino-1,2-propanediol and maximum 24 mg/ml of (*S*)-3-*tert*.-butylamino-1,2-propanediol can be determined by this method.

4. Conclusions

A new method for the enantioseparation of 3-*tert*.-butylamino-1,2-propanediol by HPLC-ELSD has been developed and validated. This amino alcohol combines two characteristics that make UV detection particularly insensitive: it possess very poor UV-absorbance properties and a UV-absorbing mobile phase is required for its enantioseparation. Using ELSD, LOQs of 26 and 48 μg/ml could be obtained for (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol, respectively. Therefore, ELSD can be regarded as a useful alternative to UV detection in this case. As many parameters of the ELSD instrument are able to influence the repeatability of the response, one of the enantiomer was selected as an internal standard for the quantitation of the other one and a RSD value around 0.8% could be obtained. The enantiomeric excess (e.e.) of (*S*)-3-*tert*.-butylamino-1,2-propanediol was measured from 0 to 94%. A technique for the improvement of the e.e. range to 0–99.8% is also proposed. This method could be used as a starting point for the enantioseparation of other aliphatic amino alcohols.

Acknowledgements

Many thanks are due to DSM Research (Geleen, The Netherlands) for providing us with the instrumentation and for financial support.

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