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Determination of the enantiomers of 3-*tert*.-butylamino-1,2-propanediol by high-performance liquid chromatography using mass spectrometric detection

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Abstract

The chiral synthesis of β-blockers such as (*S*)-timolol requires a sensitive analytical method for the enantioseparation of its intermediate, 3-*tert*.-butylamino-1,2-propanediol, in the ng/ml range. The method developed is based on on-line normal-phase LC–MS–MS using a chiral stationary phase and an atmospheric pressure chemical ionization (APCI) interface. The MS detection of 3-*tert*.-butylamino-1,2-propanediol was first optimized with a pneumatically-assisted electrospray interface (ionspray). The APCI interface was then selected for LC–MS–MS because of the incompatibility of electrospray with *n*-hexane. The method was validated for both enantiomers in the 25–500 ng/ml concentration range.

Keywords: Enantiomer separation; Butylaminopropanediol; Amino alcohols

1. Introduction

3-*tert*.-Butylamino-1,2-propanediol (Fig. 1) is an intermediate for the industrial synthesis of (*S*)-timolol, a β-blocker used for the treatment of various cardiovascular disorders such as hypertension, angina pectoris and cardiac arrhythmia. In order to separate and quantify the (*S*)-enantiomer of this intermediate at the ng/ml range in bulk form, a sensitive analytical method is necessary. However, as the other amino alcohols, 3-*tert*.-butylamino-1,2-propanediol possesses low UV-absorption properties. Many analytical methods described for amino alcohols in literature involve pre- or post-derivatization [1–3]. Another method was developed using liquid chromatography–evaporative light scattering detection (LC–ELSD) [4]. That method was faster than the previous ones because no derivatization was involved. A limit of quantitation around 50 μg/ml was obtained for (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol. Nowadays, LC coupled to mass spectrometric (MS) detection has become widely used for both identification and quantitation of drugs at very low concentrations, in bulk form or in biological fluids [5–13]. The use of atmospheric pressure ionization (API) interfaces, based on electro-

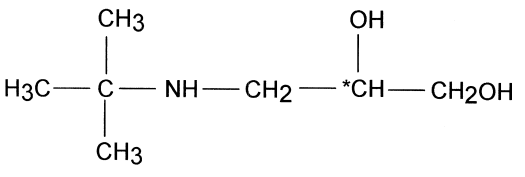

Fig. 1. Chemical structure of 3-*tert*.-butylamino-1,2-propanediol.

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spray ionization (ESI) or atmospheric pressure chemical ionization (APCI), allows the direct coupling of LC to MS. Although pneumatically-assisted electrospray (ionspray) is only compatible with LC flow-rates lower than 200 μl/min, APCI can manage flow-rates in the range of 1 ml/min without splitting the effluent [14–16]. Moreover, APCI is compatible with organic solvents such as *n*-hexane used in normal-phase LC but ESI is not.

The method reported in this paper is based on the normal-phase LC separation of 3-*tert*.-butylamino-1,2-propanediol enantiomers and their subsequent MS–MS detection using an APCI interface. The MS detection of the enantiomers was first optimized using continuous infusion of the compounds dissolved in methanol–water–formic acid (50:50:0.1, v/v) into MS–MS with an ionspray interface. The LC–APCI-MS–MS coupling was then performed using samples dissolved in the LC mobile phase and the method was validated in terms of linearity, precision and accuracy. LC–MS–MS was found to be $5 \cdot 10^3$-fold more sensitive than LC–ELSD [4].

2. Experimental

2.1. Chemicals

(*S*)- and (*R,S*)-3-*tert*.-butylamino-1,2-propanediol were kindly offered by DSM (Venlo, The Netherlands). Methanol, ethanol, *n*-hexane, formic acid 98–100% and water were obtained from Merck (Darmstadt, Germany). Diethylamine 99% was from Fluka. All chemicals used were of analytical grade. Nitrogen (99.999%) was purchased from Air Liquide (Milmort, Belgium).

Sample solutions for the ESI-MS optimization were prepared by dissolving (*R,S*)-3-*tert*.-butylamino-1,2-propanediol in methanol–water–formic acid (50:50:0.1, v/v/). Sample solutions for LC–APCI-MS–MS were made in the mobile phase.

2.2. Instrumentation and methods

The LC system was a Model 1100 series liquid chromatograph equipped with a binary pump, a vacuum degasser, a thermostated column compartment and an autosampler, all from Hewlett-Packard (Palo Alto, CA, USA). The LC separations were performed at 25°C on a Chiralpak AS column (10 μm, 250 mm×4.6 mm I.D.) from Daicel (Tokyo, Japan). The mobile phase consisted of *n*-hexane–ethanol–formic acid–diethylamine (90:10:0.2:0.2, v/v). The mobile phase was degassed for 15 min in an ultrasonic bath before use. The flow-rate was 1.0 ml/min.

MS detection was carried out using a Perkin-Elmer (PE) Sciex API 365 triple quadrupole instrument (Thornhill, Toronto, Canada) equipped with an APCI interface. The heated nebuliser temperature was 200°C and the nebulizer gas (air) flow-rate was 1.02 l/min. The curtain gas (N_2) flow-rate was 0.95 l/min at 60 p.s.i. (1 p.s.i.=6894.76 Pa). The mass spectrometer was set to generate and to select positive pseudo-molecular ions at m/z 148 for 3-*tert*.-butylamino-1,2-propanediol (corona discharge 2 μA). The MS–MS fragmentation of the pseudo-molecular ion was achieved with a collision energy of 29 eV (collision gas: N_2).

LC–MS–MS system control and collection of the data were performed with an Apple Macintosh computer (Austin, TX, USA) equipped with a version 1.3 software from PE Sciex.

3. Results and discussion

3.1. Selection of LC conditions

The best enantioseparation of 3-*tert*.-butylamino-1,2-propanediol was obtained in normal-phase LC on a Chiralpak AS column which consists of amylose tris[(*S*)-methylbenzylcarbamate] coated on silicagel [4]. The enantioresolution (R_s) was 2.65. The mobile phase constituents were selected in order to be compatible with MS detection. Indeed, organic solvents such as *n*-hexane and ethanol used in normal-phase LC are volatile and can be used in APCI-MS. Diethylamine is reported as a suitable buffer constituent for LC–MS at a 0–10 m*M* concentration range [5]. However, due to its high proton affinity, a higher concentration of diethylamine can suppress ion production. Formic acid was selected as an acidic reagent improving the interaction of the enantiomers with the stationary phase and increasing retention. It might form an ion pair with 3-*tert*.-butylamino-1,2-

propanediol. On the other hand, it is commonly used at low concentration in MS as a volatile reagent which promotes ionization. Moreover, the vaporization of a neutral pair in the APCI source could be favored compared to charged species. Formic acid could form a ion pair with diethylamine as well, minimizing the possible ion suppression effect of that compound and improving sensitivity.

3.2. Optimization of the MS detection

The MS detection of (*R,S*)-3-*tert*.-butylamino-1,2-propanediol was first investigated by direct introduction of this compound in methanol–water–formic acid (50:50:0.1, v/v) into the MS–MS system with a pneumatically-assisted electrospray interface (ionspray) in the positive mode. This interface can manage the low flow-rate (10 μl/min) generated by the infusion syringe. Parameters such as orifice voltage, ring voltage and flow of nebulizer gas were optimized in order to obtain the protonated pseudo-molecular ion of 3-*tert*.-butylamino-1,2-propanediol in MS1. The full-scan mass spectrum of the racemic compound is illustrated in Fig. 2. The positive pseudo-molecular ion is represented by *m/z* 148 whereas its ^{13}C isotope appears at *m/z* 149.

The MS–MS fragmentation of both enantiomers gave the same mass spectrum. Fig. 3 shows the product ion mass spectrum of (*R,S*)-3-*tert*.-butylamino-1,2-propanediol. The collision energy was set to the value giving rise to the maximum fragment ion intensity with a minimum pseudo-molecular ion response left. In order to favor selectivity, the fragment ion with the highest *m/z* ratio (*m/z* 92) was selected for further MS detection in the selective reaction monitoring (SRM) mode. As SRM is based

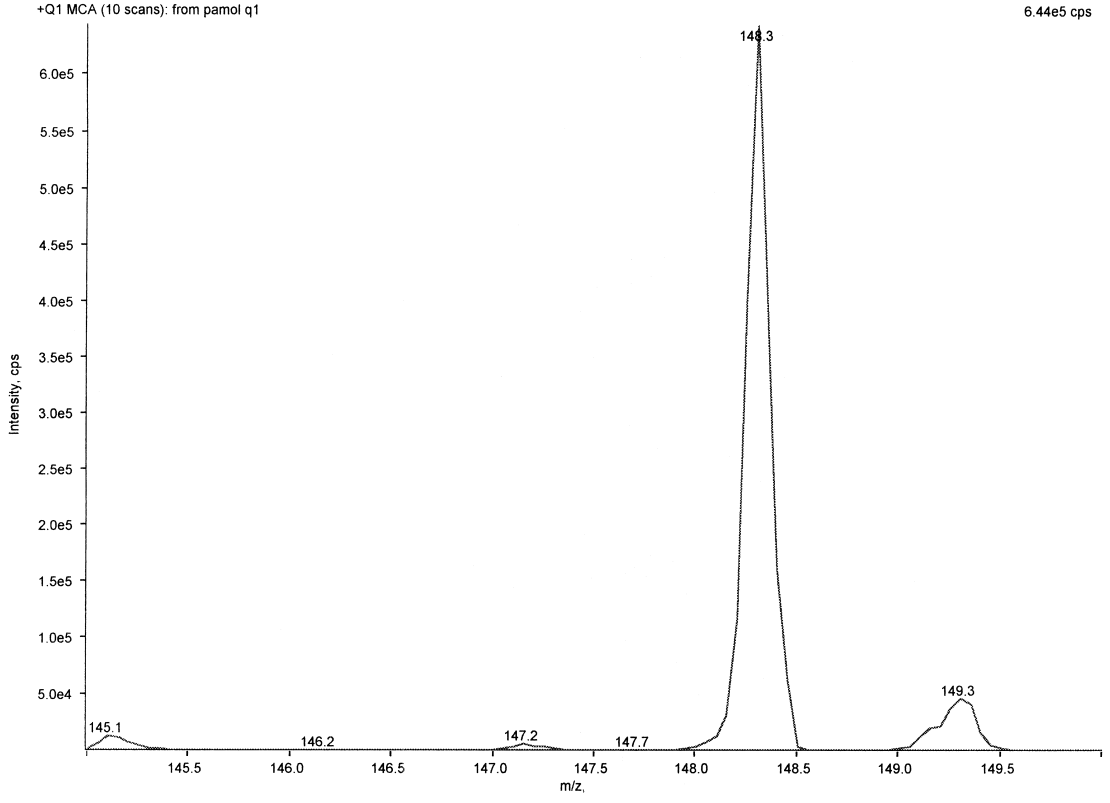

Fig. 2. Full-scan mass spectrum of racemic 3-*tert*.-butylamino-1,2-propanediol (10 μg/ml) obtained by continuous infusion of the analyte in MS, illustrating the base peaks *m/z* 148.3 and *m/z* 149.3 as the protonated pseudo-molecular ion and the ^{13}C isotope of 3-*tert*.-butylamino-1,2-propanediol, respectively, (cps) counts per second.

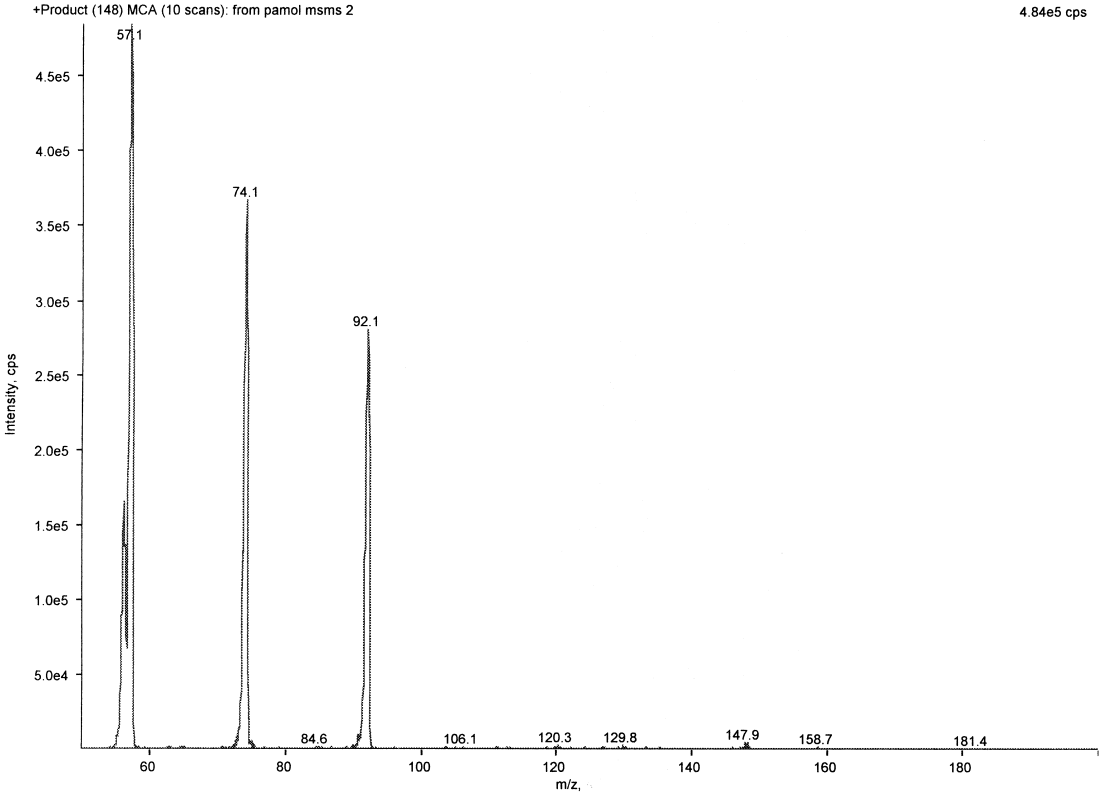

Fig. 3. Product ion mass spectrum of the protonated pseudo-molecular ion of (*R*,*S*)-3-*tert*.-butylamino-1,2-propanediol (10 μg/ml) obtained by continuous infusion of the analyte in MS, (cps) counts per second.

on the transition involving a pseudo-molecular ion and a specific fragment ion, high detection selectivity as well as high signal-to-noise ratios can be obtained providing a clean ion chromatogram.

The normal-phase LC–MS–MS coupling was then achieved using APCI as an interface with the same voltages as applied above. The corona discharge and the ion source temperature were optimized in order to get the maximum ionization and a stable spray. The SRM ion chromatogram of (*R*,*S*)-3-*tert*.-butylamino-1,2-propanediol is illustrated in Fig. 4.

3.3. Validation

The method for the determination of (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol by LC–MS–MS was validated over 3 days ($k=3$) for both enantiomers.

3.3.1. Linearity

The analysis of the response function of (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol in the 25–500 ng/ml concentration range corresponded to a weighted linear model (weight: $1/x$), where y represents the peak area and x the concentration in ng/ml. The calibration curves of both enantiomers were constructed at six concentration levels and three independent determinations ($n=3$) were performed at each concentration. The equations are reported in Table 1. The linearity of the response as a function of the concentration was demonstrated by a determination coefficient (r^2) of 0.9989 and 0.9996 for (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol, respectively.

3.3.2. Limits of detection and quantitation

The method was validated down to 25 ng/ml for

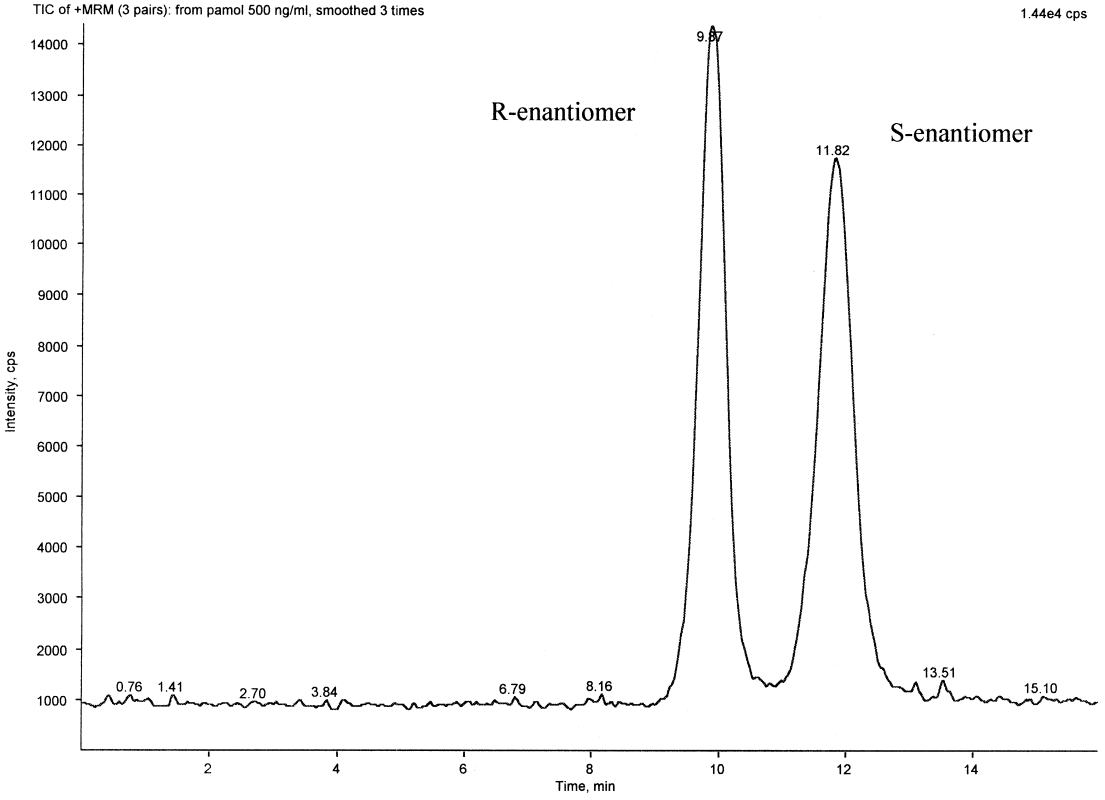

Fig. 4. SRM ion chromatogram obtained after LC–MS–MS analysis of (*R*,*S*)-3-*tert*.-butylamino-1,2-propanediol (500 ng/ml), (cps) counts per second. (*R*)-3-*tert*.-Butylamino-1,2-propanediol elutes first at 9.87 min whereas the (*S*)-enantiomer elutes at 11.82 min.

each enantiomer. However, when the limits of detection (LODs) and quantitation (LOQs) were determined as the concentration giving signal-to-noise ratios of 3 and 10, respectively, LODs and LOQs could be found at 5 and 16 ng/ml for the (*R*)-form and 7 and 22 ng/ml for the (*S*)-form, respectively.

3.3.3. Precision

The precision of the method was determined by measuring repeatability and intermediate precision (day-to-day precision) for the amount of (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol found using the linear regression. Precision was measured over 3 days ($k=3$) at three concentration levels. As can be seen in Table 1, the mean values for repeatability and intermediate precision were 1.3 and 1.9% for the (*R*)-enantiomer and 1.6 and 2.0% for the (*S*)-enantiomer, respectively.

3.3.4. Accuracy

Method accuracy was assessed for both enantiomers at three concentration levels ($k=3$, $n=18$) using the linear regression: recoveries (ratio between the analyte concentration found and the concentration applied in samples) were calculated with a confidence interval (CI) ($P>0.05$). According to the validation guidelines reported in the literature, the LC–MS–MS procedure could be considered accurate within the concentration range investigated with recoveries included in the interval 98–102% [17,18]. Mean recoveries of 99.6 and 99.2% with CIs of 1.2

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

	(*R*)-Enantiomer	(*S*)-Enantiomer
Weighted linear model ($1/x$)		
Calibration range (ng/ml)	25–500	25–500
Calibration points	6	6
Equation, where y=(*S*)-form area and x=(*S*)-form concentration	$y=65.5819x+135.8710$	$y=64.0239x-4.7662$
Coefficient of determination (r^2)	0.9989	0.9996
Accuracy ($k=3$, $n=18$)		
Mean recovery±CI (%) at 50 ng/ml	98.3±1.0	98.3±1.5
Mean recovery±CI (%) at 250 ng/ml	102.0±0.8	100.8±1.2
Mean recovery±CI (%) at 500 ng/ml	98.4±1.9	98.4±1.6
Repeatability ($k=3$, $n=6$, RSD, %)		
50 ng/ml	1.0	1.8
250 ng/ml	1.0	1.1
500 ng/ml	2.0	1.9
Intermediate precision ($k=3$, $n=18$, RSD, %)		
50 ng/ml	1.2	2.2
250 ng/ml	1.6	2.0
500 ng/ml	2.8	1.9

and 1.4% were obtained for (*R*)- and (*S*)-3-*tert*.-butylamino-1,2-propanediol, respectively (Table 1).

4. Conclusions

A sensitive method based on normal-phase LC–MS–MS has been developed for the enantioseparation and the quantitation of racemic 3-*tert*.-butylamino-1,2-propanediol in bulk form at the low quantitation limit of 20 ng/ml. The procedure was validated in terms of linearity, precision and accuracy. Seeing that 3-*tert*.-butylamino-1,2-propanediol, like other amino alcohols, possesses very poor UV-absorbance properties and requires a UV-absorbing mobile phase for its enantioseparation, MS detection represents a very powerful alternative to UV for the quantitation of aliphatic alcohols.

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