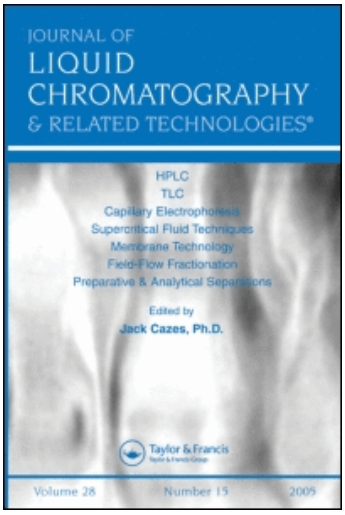


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Enantioanalysis of Tertatolol in Plasma and Pharmaceutical Formulations with Immobilized Polysaccharide-Derived HPLC Chiral Column at Nano-detection Level

Mohamed Hefnawy[a]; Abdulrhman Al-Majed[a]; Aymen Al-Suwailem[a]
[a] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

**To cite this Article** Hefnawy, Mohamed , Al-Majed, Abdulrhman and Al-Suwailem, Aymen(2009) 'Enantioanalysis of Tertatolol in Plasma and Pharmaceutical Formulations with Immobilized Polysaccharide-Derived HPLC Chiral Column at Nano-detection Level', Journal of Liquid Chromatography & Related Technologies, 32: 13, 1934 — 1952
**To link to this Article: DOI:** 10.1080/10826070903091654
**URL:** http://dx.doi.org/10.1080/10826070903091654

*Journal of Liquid Chromatography & Related Technologies*®, 32: 1934–1952, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903091654

# Enantioanalysis of Tertatolol in Plasma and Pharmaceutical Formulations with Immobilized Polysaccharide-Derived HPLC Chiral Column at Nano-detection Level

**Mohamed Hefnawy, Abdulrhman Al-Majed, and Aymen Al-Suwailem**

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia



**Abstract:** A sensitive and selective high performance liquid chromatography (HPLC) method has been developed for the simultaneous determination of tertatolol enantiomers in plasma and pharmaceutical formulations. Enantiomeric resolution was achieved on cellulose tris(3,5-dichlorophenylcarbamate) immobilized onto 5 μm, spherical porous silica chiral stationary phase (CSP) known as Chiralpak IC with UV detection set at 254 nm. The mobile phase which consisted of n-hexane-ethanol-triethylamine (60:40:0.1%, v/v/v) was used with a flow rate of 0.3 mL/min. Bentazepam was chosen as internal standard to guarantee a high level of quantitative performance. The assay involved the use of solid phase extraction procedures for human plasma samples prior to HPLC analysis; $C_{18}$ cartridges gave good recoveries for both enantiomers without any interference. The stability of tertatolol enantiomers under different degrees of temperature was studied. The result shows that the drug was stable for at least 7 days at 70°C. The method was validated for its linearity, accuracy, precision, and robustness. The calibration curves in plasma were linear over the range of 25–1000 ng/mL for each enantiomer with a detection limit of 3 ng/mL. The mean relative standard deviation (RSD) of the results of within-day precision and accuracy of the drug were $\leq 2$. There was no significant difference ($P > 0.05$) between inter-and intra-day studies for each enantiomer, which ensure the reproducibility of the assay method. The percentage recovery for both enantiomers from plasma

Correspondence: Mohamed Hefnawy, Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia. E-mail: mhefnawy2003@yahoo.com

was in the range of 91.3–98.7% at 75−800 ng/mL level for each enantiomer. The overall recoveries of tertatolol enantiomers from pharmaceutical formulation were in the range of 98–101% with %RSD ranged in 2.74–2.9%. The assay method is supposed to be suitable as therapeutic drug monitoring and chiral quality control for tertatolol formulations by HPLC.

**Keywords:** Chiralpak IC, Human plasma, Pharmaceutical formulations, Solid phase extraction, Tertatolol



## INTRODUCTION

Amino alcohol drugs, such as beta-blockers are chiral hydroxyl-amine containing compounds. Some of the clinical uses of these drugs are treatment of hypertension, angina pectoris, supraventircular, and ventricular arrhythmias and also, they are known to reduce the intensity of migraine headaches.[1] Most of amino alcohol drugs are marketed as a racemic mixture. Given the fact that these drugs have many side effects such as gastrointestinal disturbances, dizziness, depression, asthmatic wheezing (which is most likely induced by the (+)-enantiomer); therefore, there is undoubtedly a great need to develop quick methods for their enantiomeric resolution.[1] Tertatolol, (±)-hydroxy-2-tert-butylamino-3-propyloxyl)-8-thiochromane, is a powerful, long acting, and non-cardioselective $\beta$-blocker without partial agonistic activity.[2] Tertatolol differs from other $\beta$-blockers in that it increases renal blood flow in both hypertensive and normotensive patients.[3] Tertatolol is marketed as a racemic mixture of the two optical isomers. The (−)-enantiomer is about 100 fold more potent than the (+)-enantiomer; the $\beta$-adrenoceptor blockade and renal vasodilatation induced by tertatolol have been demonstrated to be stereospecific for the (−)-enantiomer.[4] Several analytical techniques for the determination of tertatolol have been reported. The GC-MS technique has been used for the determination of tertatolol in human plasma and urine.[5] Musch et al. reported the HPLC method for the determination of $\beta$-blockers in plasma using solid phase extraction.[6] A gradient high performance liquid chromatography for simultaneous determination of thirteen $\beta$-blockers and one metabolite using photodiode-array UV detection was published.[7]

Direct and indirect chiral methods for analysis of tertatolol have been reported. An indirect method involves solid phase extraction followed by derivatization with S-(+)-naphthylethylisocyanate to form the urea derivative, which was separated by RP-HPLC.[8] Direct methods used chiral stationary phase consisting of the $\beta$-cyclodextrin bonded phase,[9] or 3,5-dichlorophenyl-L-carbamate chiral column.[10] Reviewing the literature revealed that up to the present time, nothing has been

published concerning analysis of tertatolol enantiomers utilizing a Chiralpak IC column.

Molecular chirality plays an important role in the biological processes by involving stereospecific interaction of enantiomers such as distribution rates, metabolism, excretion, antagonistic actions, enzymes interactions, and receptor recognition.[11] That is why enantiomers show different pharmacological effects in the biological system.[12–15] Nowadays, the chiral separation has become an essential and urgent need in pharmaceutical industries to provide safe medication to the society. Among many analytical tools for enantiomeric separation, chromatography is the best technique due to its wide range of applications, efficiency, reproducibility, high speed, resolution, and sensitivity. Many modalities of chromatography such as thin layer chromatography (TLC), high performance liquid chromatography, and capillary electrochromatography, have been used for chiral drugs separation, but (HPLC) has a wide range of applications due to the availability of different chiral stationary phases, besides good efficiency, and low limits of detection.[16]



Polysaccharide derivatives, being coated or immobilized on silica matrix, have become the first and broadest choice of selectors to be used as chiral stationary phases (CSPs) for both liquid and supercritical fluid chromatography. Polysaccharide-derived CSPs are currently playing an essential role in both analytical and preparative separation of enantiomers.[17–20] For over two decades the coated polysaccharide-derived supports were routinely used by most of the scientific and industrial groups working in the field. Since 2004, three immobilized polysaccharide-derived CSPs have become commercially available: Chiralpak IA, Chiralpak IB, and Chiralpak IC. They are based on tris-(3,5 dimethylphenyl carbamate) of amlyose, tris-(3,5 dimethylphenyl carbamate) of cellulose, and tris-(3,5 dichlorophenyl carbamate) of cellulose, respectively.[21–24] Cellulose and amylose polysaccharide are naturally occurring polymers, which incorporate various levels of chiral information in their structure. In the early 1980s, a group of Japanese scientists came up with the idea of derivatizing them in order to enhance their chiral recognition abilities, and then coating them on a silica gel support.[25,26] This important step forward has resulted in polysaccharide derivatives that have become the first and broadest choice of selectors to be used for liquid and supercritical fluid chromatography (LC) and (SFC), for both analytical and preparative separations of enantiomers. With the motivation to further enhance the scope of application of these CSPs, various research groups have investigated possibilities of immobilizing the polysaccharide derivatives onto the chromatographic matrices (most often silica gel).[27–29] The methods used to render the polysaccharide derivatives insoluble in any mobile phase solvent are based on two general approaches: the direct covalent linkage of the derivative on the support[30–33] or the reticulation of the polysaccharide by a cross linking

reaction.[34,35] Some of the reported immobilization technologies aimed to combine both strategies.[36–39] After more than a decade of research into this challenging topic, a new series of immobilized polysaccharide-derived CSPs has become commercially available, they are Chiralpak IA, Chiralpak IB, and Chiralpak IC. Owing to their immobilized nature, these CSPs combine the benefits of the coated polysaccharide-type CSPs, namely the broad applications scope and the high preparative potential with enhanced robustness, practically unlimited solvent compatibility, and extended range of applications.

In this article, we report the development and validation of tertatolol analysis in plasma and pharmaceutical formulations using HPLC with UV detection after enantiomeric resolution on a Chiralpak IC column. The importance of the present work is the ability of the selected immobilized Chiralpak IC CSP to separate and determine tertatolol enantiomers in plasma and in pharmaceutical formulations, whereas the complementary Chiralpak IA and IB CSPs failed to separate these enantiomers. Moreover, the method is linear in the range of 25–1000 ng/mL and provides the required sensitivity for monitoring the blood level of tertatolol (70–160 ng/mL). In addition, this method should be used as chiral formulations quality control for tertatolol to check the purity of the (−)-isomer, as well as for plasma analysis. With the present broad range of available CSPs and advances in column technology, the present enanioselective HPLC can be considered as the method of choice.



## EXPERIMENTAL

### Apparatus and Reagents

Chromatography was performed on a Waters Corporation system (Milford, MA 01757 USA) consisting of a 1500 series HPLC pump and 2487 UV-Vis spectrophotometric detector. Data collection and integration was accomplished using a Dell computer. The CSP used in this study was the cellulose tris-(3,5-dichlorophenyl-carbamate), which is immobilized on 5 μm silica gel known as Chiralpak IC (250 × 4.6 mm ID), purchased from Chiral Technologies Europe (Cedex, France). The mobile phase was n-hexane-ethanol-triethylamine (60:40:0.1%, v/v/v). The mobile phase was filtered through a Millipore membrane filter (0.2 μm) from Nihon, Millipore (Yonezawa, Japan) and degassed before use. The flow rate was 0.3 mL/min with UV detection at 254 nm. (±)-Tertatolol, (−)-tertatolol, and (+)-tertatolol were purchased from RBI (Natick, MA, USA). Bentazepam was purchased from Sigma-Aldrich (St. Quentin Fallavier, France). HPLC grade n-hexane, ethanol, and analytical grade triethylamine were purchased from BDH

chemicals (Poole, UK). Analytical grade sodium hydroxide was purchased from WINLAB (UK). Deionized water was purified using a cartridge system (Picotech Water System RTP, NC, USA). Water Oasis HLB and Sep-Pak $C_{18}$, $C_8$, $C_2$, and CN cartridges were obtained from Water Corporation, (Milford, MA, USA). Human plasma was obtained from King Khalid University Hospital (Riyadh, KSA) and was kept frozen until use after gentle thawing.

## Preparation of Stock and Standard Solutions

Stock solutions containing 1 mg/1 mL of individual (−)- and (+)-tertatolol were prepared in methanol. Its purity was found to be $99.7 \pm 0.31$ by spectrophotometric measurement at 254 nm. Working standard solutions (10 μg/mL) were prepared by dilution of individual aliquots of stock solution with the same solvent. The internal standard bentazepam was prepared in methanol to give a concentration of 400 μg/mL and was further diluted with methanol to give a working solution of 40 μg/mL. The solutions were stable for at least seven days if kept in the refrigerator. Appropriate dilutions of the individual working solutions of tertatolol and internal standard were made and used for constructing the calibration curves and spiking human plasma.



## Preparation of Plasma Quality Control Samples

The quality control (QC) samples at three concentrations 75, 500, and 800 ng/mL were prepared by spiking the drug free plasma with appropriate volumes of individual (−)- and (+)-tertatolol, and stored frozen until analysis. Before spiking, the drug free plasma was tested to make sure that there was no endogenous interference at the retention time of (−)- and (+)-tertatolol, as well as the retention time of the internal standard betazepam. The QC samples were extracted with the calibration standards to verify the integrity of the method.

## Assay Method

A human plasma sample (0.3 mL) was placed into 1.5 mL Eppendrof tube and accurately measured aliquots of 7.5, 50, and 80 μL of the individual working standard (–)- and (+)-tertatolol solutions were added. Then 25 μL of internal working standard solution was added to each tube and diluted to 1 mL with methanol and sonicated for 5 minutes to give final concentrations of 75, 500, and 800 ng/mL for each tertatolol

enantiomer. Blank human plasma samples were processed in the same procedures using methanol instead of tertatolol enantiomers. Water Oasis HLB and Sep-Pak $C_{18}$, $C_8$, $C_2$, and CN cartridges and different elution solvents consisting of a different ratio of n-hexane with a different ratio of ethanol, isopropanol, tetrahydrofuran, ethyl acetate, acetonitrile, or dichloromethane, with or without a different ratio of triethylamine, have been tested for best separation of enantiomers. Cartridges were attached to a vacuum manifold (VacElute, Harbor City, CA, USA) and conditioned with four equal volumes (250 μL) of absolute methanol and two equal volumes (250 μL) of deionized water before applying the plasma samples. Care was taken that the cartridges should not run dry. Blank and spiked plasma samples were transferred into the cartridges and vacuum was applied to obtain a flow of 0.5 mL/min after the entire plasma samples had been aspirated through the cartridges; the cartridges were washed with $4 \times 250$ μL deionized water. The cartridges were then dried under vacuum for five minutes. All cartridges were eluted with $4 \times 250$ μL of 90% methanol, 10%, 1 M NaOH, then the eluted solutions were evaporated till dryness under gentle air then reconstituted by 1 mL with absolute methanol and 100 μL was injected into the HPLC system.

The absolute recoveries of each enantiomer from plasma were calculated by comparing drug peak area of the spiked analyte samples to unextracted analyte of stock solution, which has been injected directly into the HPLC system.

Calibration curves were constructed by diluting stock solutions with pooled human plasma to yield six concentrations over the range of 25–1000 ng/mL for each tertatolol enantiomer. Linear regression analysis of normalized drug/internal standard peak area ratio versus concentration gave slope and intercept data for each analyte, which were used to calculate the concentration of each analyte in the human plasma sample. The within-run and between-run precision (reported as % RSD) and accuracy (reported as % error) of the assay in plasma were determined by assaying three quality control samples over a period of three days. The concentrations represented the entire range of the calibration curve. The regression equations were used to determine the concentrations in the quality control samples.



## Preparation of Tablet Solutions

Ten prepared tablets were powdered. An accurately weighed portion equivalent to 3 mg tertatolol was transferred to a 100 mL volumetric flask diluted to the mark with methanol. The solution was sonicated for 15 minutes, centrifugated at 3000 rpm for 10 minutes. Accurately measured aliquots of the supernatant were transferred to three 5 mL volumetric

flasks to get the final concentration of tertatolol equivalent to 100, 400, and 900 ng/mL for each enantiomer.

## RESULTS AND DISCUSSION

### Optimization of the Chromatographic Conditions

The chemical structures of chiral selectors Chiralpak IA, IB, and IC are shown in Figure 1. The chemical structures of (–)-tertatolol, (+)-tertatolol, and bentazepam (IS) are shown in Figure 2. The chemical structure of both Chiralpak IC column and the drug tertatolol and its resolution is widely affected by several factors, one of the strong factors is the mobile phase used and it's components, plus the ratio of those components. The nature of mobile phase can affect enantioselectivity, retention factors, resolution degree, and some other system operating parameters, as well as the CSP stability and column life time, particularly with those based on polysaccharide derivatives.[22] Two groups of solvents, which can be distinguished with regard to the nature of the CSP, are listed in (Table 1). In principle, the solvents in the first group, denoted as "standard" solvents, can be safely applied on coated (non-immobilized) polysaccharide-based CSPs. These solvents are either non polar solvents, such as hexane and heptane, or polar solvents, like alcohols and acetonitrile. The coated CSPs, however, are not compatible with the solvents in the "non-standard" group.[22] The solvents of medium polarity, such as tetrahydrofuran (THF), ethyl acetate, and chloroform, may cause a damage of the CSP. Once immobilized, the CSP becomes compatible with the whole range of solvents (standard and non-standard) and its enantioselective capacity can be further exploited without compromising the CSP stability.[22] In this study, different solvents and different ratio of solvents with or without additive have been tested, and finally the suitable mobile phase observed is n-hexane-ethanol-triethylamine (60:40:0.1%, v/v/v), which gave good resolution

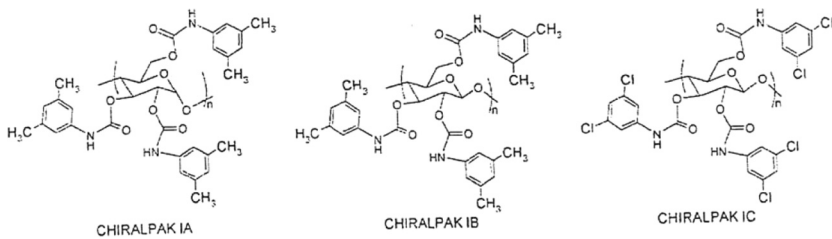


***Figure 1.*** Chemical structures of the chiral selectors.

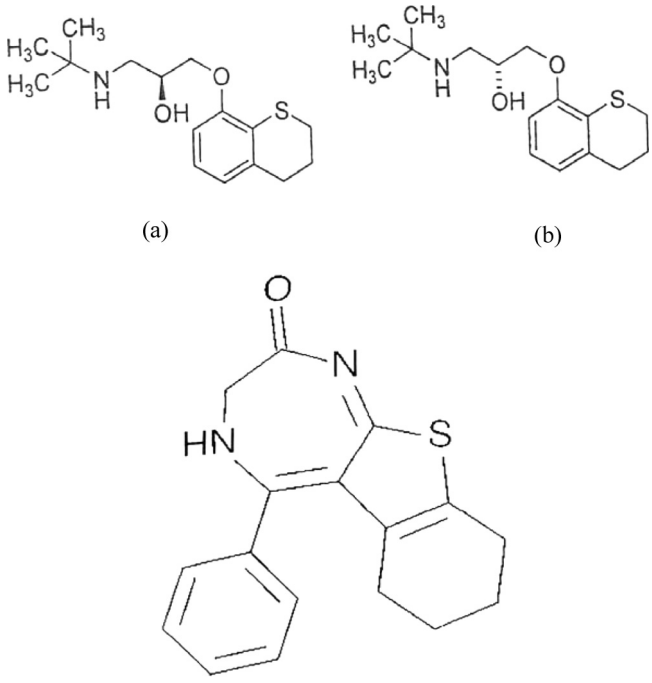


***Figure 2.*** The chemical structure of (a) (−)-tertatolol, (b) (+)-tertatolol, and (c) bentazepam.



for both enantiomers and reasonable separation factors. The importance of ethanol in this mobile phase is to improve peak shape, shortening of retention time, and enhancement of selectivity. However, a further increase in the ethanol ratio in the mobile phase causes a dramatic increase in the retention time of the drug, which may be related to hydrogen bonding between both enantiomers of tertatolol and CSP. On the

***Table 1.*** Classification of solvents according to their nature towards CSP

| Standard | Non-standard |
|---|---|
| Alkane (typically hexane, heptane) | Chlorinated solvents (typically chloroform, dichloromethane). |
| Alcohol (typically ethanol, methanol). | Esters (typically ethyl or methyl acetate). |
| Acetonitrile | Acetals (typically methylal, ethylal) ketones (typically acetone). Aromatics (typically toluene). |

other hand, TEA (0.1%) is used in this mobile phase to play an essential role for the separation of tertatolol (which is a basic drug), through suppression of the deleterious effect of residual free silanols on the silica surface, which consequently improve the peak symmetry, resolution, and selectivity.[22]

The mechanisms behind separation of tertatolol using Chiralpak IC columns are not fully understood. However, it is known that the interactive forces such as hydrogen bonding, $\pi$-$\pi$ interactions, Van der waal forces, and steric effect are responsible for the chiral resolution using these CSPs. However, native cellulose and amylose derivatives (non-immobilized) are not efficient chiral selectors because of the insufficient optical resolving power.[40]

## Applications to Spiked Human Plasma



In the course of developing a solid phase extraction (SPE) procedure for plasma sample cleanup, several types of cartridges were investigated (Water Oasis HLB and Sep-Pak $C_{18}$, $C_8$, and cyanopropyl). The cyanopropyl cartridge showed interference endogenous plasma peaks at the retention time of the analytes. An octyl ($C_8$) SPE column was also found to be unacceptable due to low recoveries (<60%) for tertatolol enantiomers and internal standard. Oasis HLB cartridges showed recoveries in excess of 70%, whereas an octadecyl ($C_{18}$) SPE column gave high recoveries for tertatolol enantiomers and internal standard (more than 90%), while at the same time removing endogenous interference. Figures 3a and b show chromatograms of a blank plasma sample and a calibration sample, respectively.

## Linearity, Precision, and Accuracy

The linear regression analysis of (−)- and (+)-tertatolol was constructed by plotting the peak area ratio of each enantiomer to the internal standard (Y) versus analyte concentration (ng/mL) in the spiked plasma sample (X). The calibration curve was linear in the range of 75–800 ng/mL for each enantiomer, with correlation coefficient (r) of more than 0.998 for (−)-enantiomer and more than 0.997 for (+)-enantiomer. A typical calibration curve has the regression of $Y = 0.0017\ x - 0.0030$ for (−)-enantiomer and $Y = 0.0016\ x - 0.0024$ for (+)-enantiomer (Table 2). A summary of the accuracy and precision results are given in (Table 3). The acceptances criteria (within-run and between-run %RSD of < 15% and accuracy between 85 and 115%) were met in all cases. The precision and accuracy of the method were determined by using plasma samples spiked at three levels (Table 3). The data

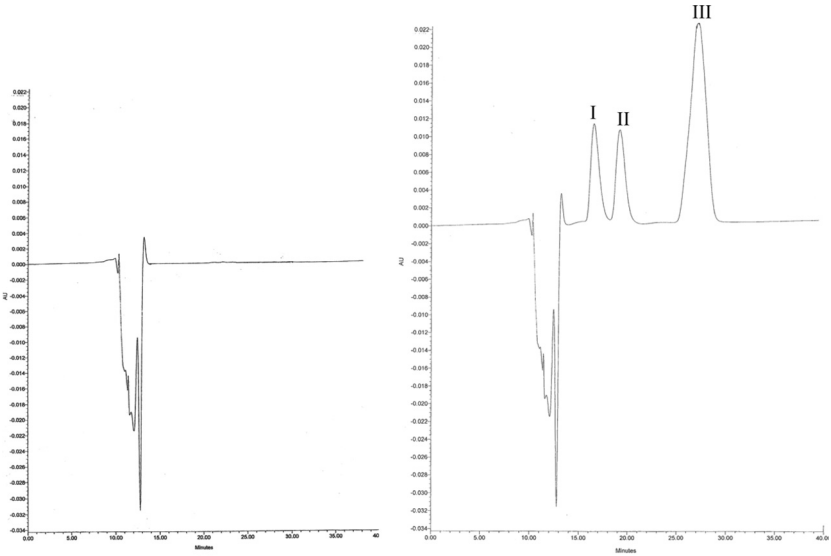


***Figure 3.*** Chromatograms of (a) Blank human plasma and (b) Spiked with 75 ng/mL of (−)-tertatolol (I), (+)-tertatolol (II), and 1000 ng/ml bentazepam (III).

indicates that within-run precision and accuracy (N = 10) as expressed by % RSD and % error were 0.9–2.0% and 1.3–6.0%, respectively, for (–)-tertatolol and 0.9–1.8% and 2.3–7.9%, respectively, for (+)-tertatolol. The between-run precision and accuracy (N = 10) expressed by % RSD and % error were 0.9–2.1% and 2.1–8.4% for (−)-tertatolol and 0.9–1.8% and 3.5–7.7%, respectively, for (+)-tertatolol.

***Table 2.*** Validation parameters for the determination of tertatolol enantiomers using the proposed method in spiked human plasma

| Parameters | (−)-Tertatolol | (+)-Tertatolol |
|---|---|---|
| Concentration range ng/mL | 25 – 1000 | 25 – 1000 |
| Intercept (a) | $-3.00 \times 10^{-3}$ | $-2.40 \times 10^{-3}$ |
| Slope (b) | $1.70 \times 10^{-3}$ | $1.60 \times 10^{-3}$ |
| Correlation coefficient (r) | 0.998 | 0.997 |
| $S_{y/x}$ | $1.90 \times 10^{-3}$ | $1.78 \times 10^{-3}$ |
| $S_a$ | $7.08 \times 10^{-7}$ | $6.50 \times 10^{-7}$ |
| $S_b$ | $3.90 \times 10^{-6}$ | $3.70 \times 10^{-6}$ |
| LOD (ng/mL)[a] | 3 | 3 |
| LOQ (ng/mL) | 25 | 25 |

[a]S/N = 3.

***Table 3.*** Accuracy and precision data for tertatolol enantiomers in spiked human plasma

| Analyte | Actual concentration ng/mL | Experimental concentration ng/mL | Recovery (%) | RSD (%)[b] | Error (%)[c] |
|---|---|---|---|---|---|
| Within-day[a] | | | | | |
| (−)-tertatolol | 75 | 70.5 ± 1.4 | 94.0 | 2.0 | 6 |
| | 500 | 456.5 ± 6.2 | 91.3 | 1.3 | 8.7 |
| | 800 | 789.6 ± 6.8 | 98.7 | 0.9 | 1.3 |
| (+)-tertatolol | 75 | 70.2 ± 1.3 | 93.6 | 1.8 | 6.4 |
| | 500 | 460.5 ± 6.0 | 92.1 | 1.3 | 7.9 |
| | 800 | 781.6 ± 7.1 | 97.7 | 0.9 | 2.3 |
| Between-day[a] | | | | | |
| (−)-tertatolol | 75 | 71.5 ± 1.5 | 95.3 | 2.1 | 4.6 |
| | 500 | 458.0 ± 6.2 | 91.6 | 1.3 | 8.4 |
| | 800 | 783.2 ± 6.9 | 97.9 | 0.9 | 2.1 |
| (+)-tertatolol | 75 | 71.0 ± 1.3 | 94.7 | 1.8 | 5.3 |
| | 500 | 461.5 ± 6.2 | 92.3 | 1.3 | 7.7 |
| | 800 | 772.0 ± 7.1 | 96.5 | 0.9 | 3.5 |

[a]Mean ± SD n = 10.
[b]Expressed as %RSD = (SD/Mean) × 100.
[c]Calculated as [(measured concentration − actual concentration)/actual concentration] × 100.



## Selectivity

The analytical figure of merit for this method is shown in (Table 4). (−)- and (+)-tertatolol were well separated under the HPLC conditions

***Table 4.*** Chromatographic parameter data for tertatolol enantiomer and bentazepam as internal standard

| Analyte | Rs[a] | a[b] | k[c] | $T_R$[c] |
|---|---|---|---|---|
| (−)-tertatolol | 4.5 | 1.7 | 0.41 ± 0.01 | 16.3 ± 0.10 |
| (+)-tertatolol | 8.8 | 2.2 | 0.73 ± 0.01 | 20.3 ± 0.08 |
| Bentazepam | *d* | *d* | 1.64 ± 0.03 | 27.9 ± 0.18 |

[a]$Rs = (t_2 - t_1)/0.5(w_2 + w_1)$, where $t_2$ and $t_1$ are the retension of the second and the first peaks while $wb_2$ and $wb_1$ are the half peak width of the second and first peaks.
[b]Separation factor, calculated as $k_2/k_1$ where $k = (tr - to)/to$, where tr is the retension of analyte and to is the retension of solvents.
[c]$T_R$ is the retension time, mean ± SD, n = 10.
[d]Not calculated.

applied. Retention times were 16.3 and 20.3 for (−)- and (+)-tertatolol, respectively. No interferences were observed in drug free human plasma samples (Figures 3a and b). Otherwise, there are no peaks detected at the retention time of the individual tertatolol enantiomer and of internal standard bentazepam. Excipients commonly coformulated with the studied drug such as cellulose, magnesium stearate, calcium hydrogen

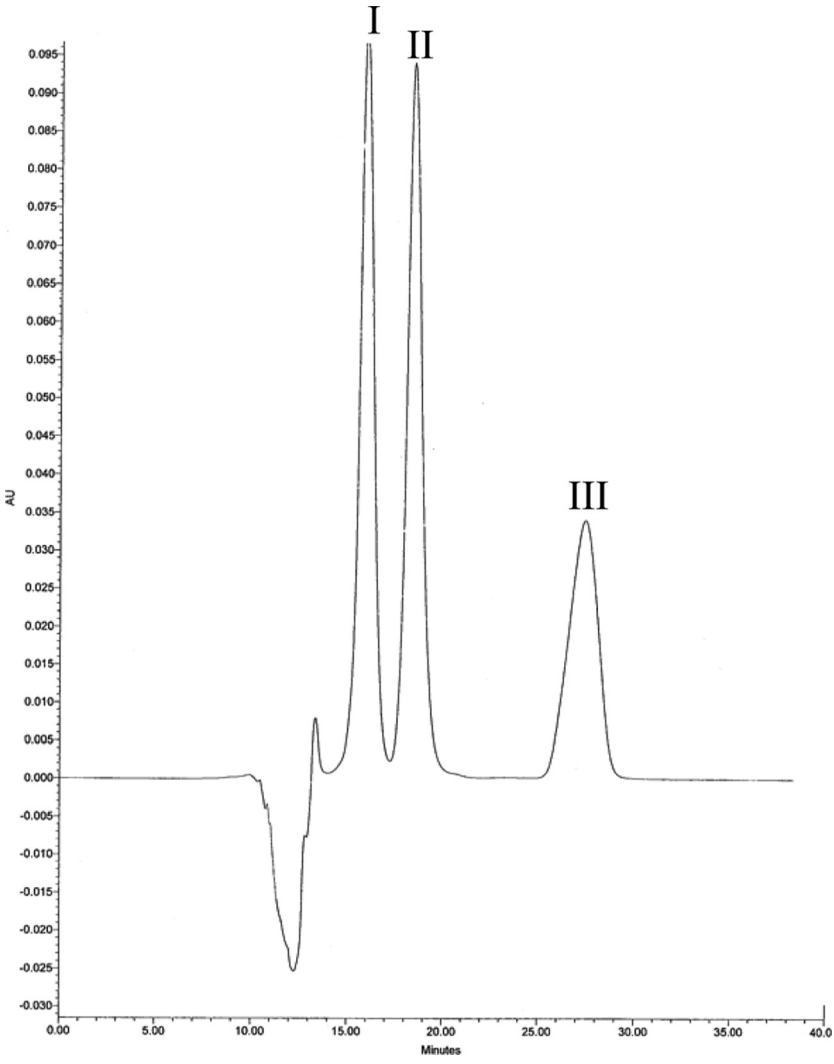


***Figure 4.*** Chromatogram of 400 ng/mL of (−)-tertatolol (I), (+)-tertatolol (II), and 1000 ng/mL bentazepam (III) recovered from tertatolol tablets.

phosphate, starch, silicone dioxide, also did not interfere with the determination of tertatolol enantiomers, indicating the high selectivity of the method (Figure 4).

## Robustness

The optimum HPLC conditions set for this method have been slightly modified for samples of tertatolol as a means to evaluate the method robustness. The small changes made in day (time), wavelength, flow rate, and temperature, ensure that the % recoveries of tertatolol enantiomers were good under all conditions, and remain unaffected by small changes of the experimental parameters (Tables 5 and 6). Variation in the experimental parameters, as well as carrying the experiment at room temperature, provided an indication of its reliability during normal use and concluded that the method conditions were robust.



## Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) for each enantiomer were 3 ng/mL and 25 ng/mL, respectively. The good

***Table 5.*** Stability of tertatolol enantiomers at various experimental conditions

| Analyte | QC Sample ng/mL | Stability condition | % Recovery ± SD |
|---|---|---|---|
| (−)-tertatolol | 75 | 4 h at room temperature. | 94.0 ± 6.3 |
| | | 3 freeze-thaw cycles | 99.1 ± 6.3 |
| | | 48 h at room temperature | 96.1 ± 7.3 |
| | 500 | 4 h at room temperature | 95.1 ± 3.4 |
| | | 3 freeze-thaw cycles | 98.7 ± 3.4 |
| | | 48 h at room temperature | 95.9 ± 4.3 |
| | 800 | 4 h at room temperature | 93.2 ± 2.1 |
| | | 3 freeze-thaw cycles | 99.0 ± 1.9 |
| | | 48 h at room temperature | 99.3 ± 1.4 |
| (+)-tertatolol | 75 | 4 h at room temperature | 96.1 ± 7.1 |
| | | 3 freeze-thaw cycles | 98.8 ± 6.6 |
| | | 48 h at room temperature | 96.6 ± 5.1 |
| | 500 | 4 h at room temperature | 93.9 ± 1.8 |
| | | 3 freeze-thaw cycles | 98.7 ± 4.3 |
| | | 48 h at room temperature | 96.6 ± 3.6 |
| | 800 | 4 h at room temperature | 96.1 ± 1.7 |
| | | 3 freeze-thaw cycles | 98.0 ± 2.2 |
| | | 48 h at room temperature | 99.9 ± 1.6 |

***Table 6.*** Effect of experimental parameters on the percentage recoveries of tertatolol enantiomers

| Parameters | Modification | Recovery (%) | |
|---|---|---|---|
| | | (−)-Enantiomer | (+)-Enantiomer |
| Temperature[a] (°C) | 30 | 99.1 | 98.6 |
| | 50 | 98.6 | 97.1 |
| | 70 | 96.9 | 95.4 |
| Flow rate (mL/min) | 0.2 | 97.9 | 98.2 |
| | 0.3 | 99.3 | 98.8 |
| | 0.4 | 96.9 | 97.1 |
| Wavelength (nm) | 250 | 98.1 | 98.3 |
| | 254 | 99.3 | 98.8 |
| | 258 | 97.9 | 98.1 |
| Day[b] | 1 | 99.3 | 98.8 |
| | 2 | 99.4 | 99.1 |
| | 3 | 99.0 | 98.8 |

[a]7-day stored solutions at 30, 50, and 70°C.
[b]Solutions were stored at room temperature.



linearity of the calibration graphs and the negligible scatter of experimental points are evident by the values of the correlation coefficient (r) and standard deviation (SD) (Table 2).

## Application to Pharmaceutical Formulations

The validity of the developed method was applied to various concentrations of tertatolol tablets for determining their content of tertatolol enantiomers. The values of the overall drug % recoveries and % RSD values of both enantiomers are presented in Table 7, indicating that these values are acceptable and the method is precise and accurate.

## Stability of Sample Solutions

The stability of sample solutions was tested by HPLC over a period of 7 days. The freshly prepared solution at room temperature and the 7 day stored samples at 30, 50, and 70°C were analyzed by the proposed HPLC method. The concentrations of the tertatolol enantiomers in the stored samples were calculated and compared to that present in the freshly prepared samples (Figure 5). From these results, we can conclude that there is no degradation products at elevated temperature

*Table 7.* Determination of tertatolol enantiomers in tablets

| Pharmaceutical preparation | Enantiomer | Actual concentration ng/mL | Measured concentration ng/mL | Recovery (%) |
|---|---|---|---|---|
| Tertatolol tablet[a] | (−) | 100 | 98.0 | 98.0 |
| | | 400 | 404.0 | 101.0 |
| | | 900 | 909.0 | 101.0 |
| Overall recovery (±SD) | | | | 99.9 ± 2.90 |
| RSD (%) | | | | |
| | | | | 2.9 |
| | (+) | 100 | 99.0 | 99.0 |
| | | 400 | 398.5 | 99.6 |
| | | 900 | 896.7 | 99.6 |
| Overall recovery (±SD) | | | | 99.5 ± 2.7 |
| RSD (%) | | | | |
| | | | | 2.7 |

[a]Tablets prepared in our lab.



and the drug is stable at 70°C for at least 7 days, indicating the possibility of using tertatolol samples over a period of 7 days at 70°C without degradations.

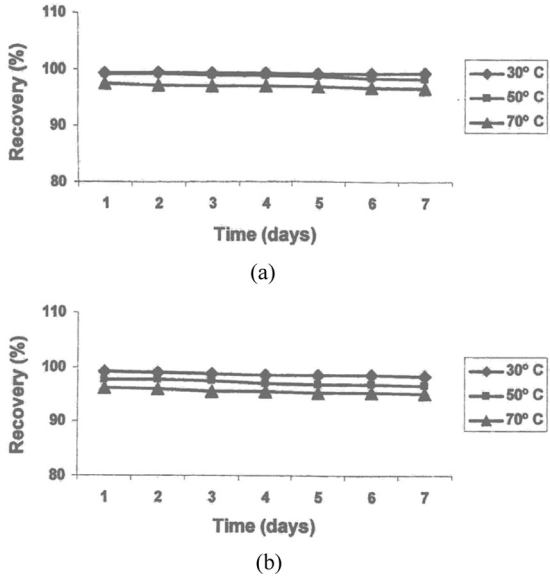


*Figure 5.* Concentration recovery vs. time (days) of (a) (−)-tertatolol and (b) (−)-tertatolol at 30°C, 50°, and 70°C.

## CONCLUSION

An enantioselective HPLC method that enables sensitive determination of both tertatolol enantiomers in plasma and in pharmaceutical formulations was developed. The method used an efficient solid phase extraction procedure for sample cleanup of plasma. The method is selective where coformulated drug excipients do not interference. With the present broad range of available CSPs and advances in column technology, the present enantioselective HPLC can be considered as the method of choice.

## ACKNOWLEDGMENT

The authors would like to thank the King Abdulaziz City for Science and Technology for their support of the Pharmaceutical Analysis Laboratory Research Programs.



## REFERENCES

1. Mark, H.; Perrone, J.; Barrett, A. Preclinical pharmacology of celiprolol: A cardioselective $\beta$-adrenoceptor antagonist and mild vasodilator. Amer. Heart J. **1991**, *121*, 677–683.
2. Laubie, M.; Schmitt, H.; Mouille, P.; Cheymol, G.; Gilbert, J.C. Beta adrenolytic and hemodynamic effects of dl (hydroxyl 2-tert-butylamino 3-propyloxyl) 8-thiochromane (S2395). Arch. Int. Pharmacodyn. Ther. **1973**, *201*, 334–346.
3. Degaute, J.P.; Nacije, R.; Abramoviez, M.; Leeman, M.; Schoutens, A.; Prost, J.F. Acute effects of tertatolol and nadolol on systemic and renal hemodynamics in patients with essential hypertention. Am. J. Hypertens. **1988**, *1*, 263S–268S.
4. Smits, J.F.; Zeegers, M.; Struyker-Bondier, H.A.J. Stereospecificity of beta-adrenoceptor blockade and renal vasodilatation by tertatolol. Arch. Int. Pharmacodyn. **1989**, *298*, 164–171.
5. Efthymiopoulos, C.; Staveris, S.F.; Weber, J.C.; Koffel, L.; Jung, J. Simultaneous quantitative determination of tertatolol and its hydroxylated metabolite in humen plasma and urine by gas chromatography-mass spectrometry. J. Chromatogr. Biomed. Appl. **1987**, *421*, 360–366.
6. Musch, G.; Buelens, T.; Massart, D.L. A strategy for the determination of beta blockers in plasma using solid-phase extraction in combination with high performance liquid chromatography. J. Pharm. Biomed. Anal. **1989**, *7*, 483–497.
7. Delamoye, M.; Duverneuil, C. F.; Paraire, P.; Mazancourt, J.C. Simultaneous determination of thirteen beta-blockers and one metabolite by gradient high-performance liquid chromatography with photodiode-array UV detection. Foren. Sci. Int. **2004**, *141*, 23–31.

8. Lave, T.; Efthymiopoulos, C.; Koffel, J.C.; Jung, L. Determination of tertatolol enantiomers in biological fluids by high-performance liquid chromatography. J. Chromatogr. **1991**, *572*, 203–210.
9. Weems, H.: Zamani, K. Resolution of terfenadine enantiomers by beta-cyclodextrin chiral stationary phase high performance liquid chromatography. Chirality. **1992**, *4*, 268–272.
10. Ali, I.; Aboul-Enein, H.Y. Enantioseparation of some clinically used drugs by HPLC using cellulose Tris (3,5-dichlorophenylcarbamate) chiral stationary phase. Biomed. Chromatogr. **2003**, *17*, 113–117.
11. Lanchote, V.L.; Takayanagui, O.M.; Mateus, F.H. Enantioselective renal excretion of albendazole metabolites in patients with neurocysticercosis. Chirality. **2004**, *16*, 520–525.
12. Eriksson, T.; Bjorkman, S.; Roth, B.; Hoglund, P. Intravenous formulations of the enantiomers of thalidomide: pharmacokinetic and initial pharmacodynamic characterization in man. J. Pharm. Pharmacol. **2000**, *52*, 807–817.
13. Fitos, I.; Simonyi, M. Investigation on the binding of tryptophan enantiomers to human serum albumin. Acta. Biochim. Biophys. Hung. **1986**, *21*, 237–245.
14. Kondo, T.; Yoshida, K.; Yoshimura, Y.; Tanayama, S. Enantioselective pharmacokinetics in animals of pazinaclone, a new isoindoline anxiolytic, and its active metabolite. Biopharm. Drug. Dispos. **1995**, *16*, 755–773.
15. Brocks, D.R.; Jamali, F. Stereochemical aspects of pharmacotherapy. Pharmacotherapy. **1995**, *15*, 551–564.
16. Ye, X.X.; Yu, X. Enantiomeric resolution of ketoprofen by HPLC using chirobiotic V CSP and vancomycin as chiral mobile phase additives. Yao Xue Xue Bao. **2003**, *38*, 211–214.
17. Zhang, T.; Kientzy, C.; France, P.; Ohnishi, A.; Kagamihara, Y.; Kurosawa, H. Solvent versatility of immobilised 3,5-dimethylphenylcarbamate of amylose in enantiomeric separations by HPLC. J. Chromatogr. A **2005**, *1075*, 65–75.
18. Yashima, E. Polysaccharide-based chiral stationary phases for high-performance liquid chromatographic enantioseparation. J. Chromatogr. A **2001**, *906*, 105–125.
19. Tachibana, K.; Ohnishi, A. Reversed-phase liquid chromatographic separation of enantiomers on polysaccharide type chiral stationary phases. J. Chromatogr. A **2001**, *906*, 127–154.
20. Mey, B.; Paulus, H.; Lamparter, E.; Blaschke, G. Enantioseparation of amfepramone (rac-diethylpropion): preparative separation of the enantiomers and enantioselective analysis. Chirality. **1999**, *11*, 772–780.
21. Ghanem, A.; Hoenen, H.; Aboul-Enein, H.Y. Application and comparison of immobilized and coated amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phases for the enantioselective separation of beta-blockers enantiomers by liquid chromatography. Talanta. **2005**, *68*, 602–609.
22. Zhang, T.; Ngugen, D.; Franco, P.; Murakani, T.; Ohnishi, A., Kurosawa, H. Cellulose 3,5-dimethylphenylcarbamate immobilized on silica: A new chiral stationary phase for the analysis of enantiomers. Anal. Chim. Acta. **2006**, *557*, 221–228.
23. Zhang, T.; Nguyen, D.; Franco, P.; Isobe, Y.; Michisita, T.; Murakani, T. Cellulose tris(3,5-dichlorophenylcarbamate) immobilised on silica: A novel

chiral stationary phase for resolution of enantiomers. J. Pharm. Biomed. Anal. **2008**, *46*, 882–891.
24. Zhang, T.; Ngugen, D.; France, P. Enantiomer resolution screening strategy using multiple immobilised polysaccharised-based chiral stationary phases. J. Chromatogr. A **2008**, *1191*, 214–219.
25. Okameto, Y.; Kawashima, M.; Hatada, K. Chromatographic resolution : XI. Controlled chiral recognition of cellulose triphenylcarbamate derivative supported in silica gel. J. Chromatogr. **1986**, *363*, 173–186.
26. Okamoto, Y.; Noguchi, J.; Yashima, E. Enantioseparation on 3,5-dimethyphenylcarbamates of polysaccharide as chiral stationary phases for high performance liquid chromatography. React. Funct. Polym. **1998**, *37*, 183–188.
27. Okamoto, Y.; Aburatani, R.; Hatada, K.; Honda, M.; Inotsume, N.; Nakano, M. Optical resolution of dihydropyridine enantiomers by high performance liquid chromatography using phenylcarbamates of polysaccharides as chiral stationary phase. J. Chromatogr. **1990**, *513*, 375–378.
28. Okamoto, Y.; Kaida, Y.; Hirose, K.; Hayashida, H.; Hatada, K. Chromatographic optical resolution on araylkylcarbamates of polysaccharides. React. Polym. **1991**, *15*, 254–255.
29. Chen, X.; Zou, H.; Zhang, Q.; Zhang, J. Synthesis of chemically bonded cellulose trisphenylcarbamate chiral stationary phases for enantiomeric separation. J. Chromatogr. Sci. **2002**, *40*, 315–320.
30. Huang, X.; Zou, H.; Chen, X.; Luo, Q.; Kong, L. Molecularly imprinted monolithic stationary phases for liquid chromatographic separation of enantiomers and diastereomers. J. Chromatogr. A **2003**, *984*, 273–282.
31. Ruta, J.; Ravelet, C.; Desire, J.; Decout, J.L.; Peyrin, E. Covalently bonded DNA aptamer chiral stationary phase for the chromatographic resolution of adenosine. Anal. Bioanal. Chem. **2008**, *390*, 1051–1057.
32. Oxelbark, J.; Gidlund, P. Investigation of a tartaric acid-based linear polyamide and dimer as chiral selectors in liquid chromatography. Chirality. **2005**, *17*, 79–84.
33. Weng, W.; Guo, H.; Zhan, F.; Fang, H.; Wang, Q.; Yao, B.; Li, S. Chromatographic enantioseparations of binaphthyl compounds on an immobilized polysaccharide-based chiral stationary phase. J. Chromatogr. A **2008**, *1210*, 178–184.
34. Mayer, S.; Briand, X.; Francotte, E. Separation of enantiomers by packed capillary electrochromatography on a cellulose-based stationary phase. J. Chromatogr. A **2000**, *876*, 331–335.
35. Francotte, E.; Huynh, D. Immobilized halogenophenylcarbamate derivatives of cellulose as novel stationary phases for enantioselective drug analysis. J. Pharm. Biomed. Anal. **2002**, *27*, 421–429.
36. Franco, P.; Senso, A.; Olivers, L.; Minguillon, C. Covalently bonded polysaccharide derivatives as chiral stationary phases in high-performance liquid chromatography. J. Chromatogr. A **2001**, *906*, 155–170.
37. Senso, A.; Franco, P.; Olivers, L.; Minguillon, C.A. Characterization of doubly substituted polysaccharide derivatives. Carbohydr. Res. **2000**, *329*, 367–376.

38. Minguillon, C.; Franco, P.; Olivers, L.; Lopez, P. Bonded cellulose-derived high-performance liquid chromatography chiral stationary phases I. Influence of the degree of fixation on selectivity. J. Chromatogr. A **1996**, *728*, 407–414.
39. Minguillon, C.; Franco, P.; Olivers, L. Bonded cellulose-derived high-performance liquid chromatography chiral stationary phases II.. Influence of the porosity of the silica gel matrix on performance. J. Chromatogr. A **1996**, *728*, 415–422.
40. Ali, I.; Naim, L.; Ghanem, A.; Aboul-Enein, H.Y. Chiral separations of piperidine-2,6-dione analogues on Chiralpak IA and Chiralpak IB columns by using HPLC. Talanta **2006**, *69*, 1013–1017.

Received December 16, 2008
Accepted January 29, 2009
Manuscript 6457