

# Chemometrical Evaluation of Metoprolol Tartarate Enantiomers Separation Applying Conventional Achiral Chromatography

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## Abstract

In this paper, the separation of metoprolol tartarate enantiomers using conventional achiral chromatography was followed employing experimental design 2<sup>4</sup>. Design was useful tool in evaluation of chromatographic behavior of enantiomers. For the separation, Supelcosil LC<sub>18</sub> 4.6 mm × 250 mm, 5 μm particle size column was used. UV detection was performed at 275 nm. Because of the large number of factors which can influence chromatographic separation, in preliminary study "one factor at a time" method was applied to define values of important factors. As outputs, retention factor, selectivity factor and resolution factors were chosen. Chromatographic behavior of investigated enantiomers was affected by acetonitrile content, chiral modifier content in the mobile phase and flow rate the most, demonstrated by obtained linear models. On the basis of results from screening of experiment, factors with strong influence on the separation, were analyzed in method optimization applying response surface methodology (RSM). The appropriate region of chromatographic behavior of metoprolol tartarate enantiomers was defined using three-D graphs and analysis of variance. The methodology proposed represents an efficient approach in resolving the problem of searching for optimal HPLC chromatographic conditions via experimental design.

**Keywords:** Metoprolol tartarate; Enantiomers; RP-HPLC; Experimental design

## Introduction

Metoprolol tartarate ((R,S)-3[4-(2-methoxyethyl) phenoxy]-1-(isopropylamino) propan-2-ol] tartarate) is a β<sub>1</sub>-adrenoreceptor antagonist (β<sub>1</sub>-blocker) widely used in the treatment of hypertension, angina pectoris and cardiac dysrhythmias [1,2].

Most β-adrenergic antagonists (β-blockers) are therapeutically used as a racemic mixture and their enantiomers demonstrate different pharmacodynamic and pharmacokinetic properties [1,2]. The both enantiomers are reducing blood pressure, but the therapeutic effects of the (S)-enantiomer is about 100 times stronger than (R)-enantiomer. The increasing demands for the production of enantiomerically pure drugs have led to enantioselective separations becoming one of the most important analytical tasks. In this context, enantioselective HPLC is one of the most powerful and widely employed separation techniques, both for analytical and preparative purposes, as well as for research in pharmaceutical and biomedical analysis.

Separation of β<sub>1</sub>-blocker enantiomers included conventional achiral chromatography with pre-column derivatisation [3,4]. Using different chiral stationary phases or chemically different chiral mobile phase additives, β-blockers has been recently separated [5-8]. Also, the spectrophotometric method for analysis of β-cyclodextrin/metoprolol tartarate inclusion complex was investigated [9].

The aim of our investigation was analysis of metoprolol tartarate enantiomers using achiral stationary phase with β-cyclodextrin as chiral additive in the mobile phase. The experimental design was applied as the best way to define chromatographic behavior of enantiomers. Analysis employed "one factor at a time" method for preliminary study, full factorial design for screening of experiment and response surface methodology (RSM) for method optimization. A literature search showed many experimental design applications in analytical method development and validation, especially in the area of separation science. Experimental design has been used for separation

optimization [10-12] and for validation in RP-HPLC method [13,14]. It was used for robustness testing in RP-HPLC method [15,16] and capillary electrophoresis [17]. The methodology proposed in this paper represents novel, efficient and easily attainable approach in resolving metoprolol tartarate enantiomers using conventional achiral chromatography.

## Experimental

### Reagents and samples

All reagents used were of an analytical grade. Methanol-gradient grade (*Merck*, Darmstadt, Germany), acetonitrile (*Merck*, Darmstadt, Germany), water-HPLC grade, triethylamine (TEA) (*Merck*, Darmstadt, Germany). Beta cyclodextrine (-CD) was obtained from *Cyclolab*, Hungary. Presolol<sup>®</sup> tablets (containing 100 mg of metoprolol taratarat) were manufactured by Hemofarm d.o.o., SCG. Working standard of metoprolol tartarate was obtained from *Hemofarm* d.o.o., SCG.

### Chromatographic conditions

The chromatographic system Hewlett Packard 1100 (*Agilent*, Technologies) consisted of a HP 1100 pump, HP 1100 UV-VIS detector and HP ChemStation integrator. Separations were performed on a Supelcosil LC<sub>18</sub> 4.6 mm × 250 mm, 5 μm particle size column.

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UV detection was performed at 275 nm. The samples were introduced through a Rheodyne injector valve with a 20 µL sample loop.

### Buffer solution

Buffer solution was prepared by adding of 2 ml of TEA to 600 ml of HPLC water, pH was adjusted in the range from 2 to 6 with glacial acetic acid.

### Solution for equilibration and storage of column

Solution was prepared in concentration of 3 mM of β-CD in water.

## Results and Discussion

In preliminary investigations, influence of different chromatographic factors on separation of metoprolol tartarate enantiomers was analyzed. As the separation was performed employing conventional achiral chromatography, beta cyclodextrine (β-CD) was added to the mobile phase as chiral modifier. Mobile phases consisted of acetonitrile and β-CD in triethylamine/glacial acetic acid buffer in different ratios. Column was stored and conditioned with solution of 3 mM of β-CD in water. In chromatography many factors can influence separation e.g., content of organic modifier in mobile phase, pH of the mobile phase, column temperature, flow rate, concentration of solute etc. In the first step of our study, pH of the mobile phase and temperature were defined using "one factor at a time" method. pH of the mobile phase (2.5; 3.0; 4.0; 5.0 and 6.0) was changed and other factors were kept at constant level. Obtained chromatograms demonstrated the best separation of enantiomers at pH 3.0 and in following investigations it remained constant. Secondly, temperature was analyzed on two levels 30°C and 40°C. The accepted separation was at 35°C column temperature.

In the second step, for the screening of experiment, full factorial design 2<sup>4</sup> was chosen. Full factorial designs at two levels are mainly used for screening, that is, to determine the influence of a number of effects on a response and to eliminate those that are not significant [18]. Selected factors and their "low" (-) and "high" (+) levels are presented in Table 1. Matrix of the experiment is given in Table 2.

As outputs (Y) capacity factors (k<sub>1</sub> (Y<sub>1</sub>)-retention factor for enantiomer R (+), k<sub>2</sub> (Y<sub>2</sub>)-retention factor for enantiomer S (-)), selectivity factor (α-Y<sub>3</sub>) and resolution factor (Rs-Y<sub>4</sub>) were analyzed. The obtained results are presented in Table 3.

In experimental design for the evaluation of influence of investigated factors, on measured response, mathematical model was applied. Often form of a mathematical model is:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + \dots + b_{N-1}x_{N-1} + b_Nx_N + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + \dots + b_{(N-1)N}x_{N-1}x_N \quad (\text{Eq. 1})$$

where y presents the estimate response, b<sub>0</sub> is the average experimental response, the coefficients b<sub>1</sub> to b<sub>N</sub> are the estimated effects of the factors considered and the extend to which these terms affect the performance of the method is called main effect. The coefficients b<sub>12</sub> to b<sub>(N-1)N</sub> are called the interaction terms. We can see that the factorial design provides information about the importance of interaction between the factors [18]. The calculating coefficients of mathematical models for outputs are presented in Table 4.

The results showed that acetonitrile content and concentration of β-CD had the biggest influence on retention factors (Y<sub>1</sub> and Y<sub>2</sub>). The flow rate and content of β-CD influenced selectivity factor (Y<sub>3</sub>) and resolution (Y<sub>4</sub>) the most. Concentration of metoprolol tartarate had

negligible influence on analyzed outputs and in further investigations it was kept constant.

In the third step of method optimization, three factors (content of acetonitrile, content of β-CD and flow rate) were analyzed in 22 experiments. Matrix of experiment for optimization is presented in Table 5. Experimental data for outputs are presented in Table 6.

On the basis of the results, outputs Y<sub>1</sub> and Y<sub>4</sub> were chosen to analyze separation. The results for others outputs gave bad coefficient of determination (≤ 0.5) and they fitted badly in the obtained model. Those results could be explained with different characters of

Factors		Levels of the investigation	
		(-)	(+)
X <sub>1</sub>	Acetonitrile (%)	9.1	14.5
X <sub>2</sub>	β-CD (mM)	5.81	8.81
X <sub>3</sub>	Flow rate (mlmin <sup>-1</sup> )	1	1.5
X <sub>4</sub>	Concentration of metoprolol tartarate (mL <sup>-1</sup> )	0.25	0.50

(-) and (+) are "low" and "high" levels

Table 1: Factor and their levels.

Number of experiments	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>
1	-	-	-	-
2	+	-	-	-
3	-	+	-	-
4	+	+	-	-
5	-	-	+	-
6	+	-	+	-
7	-	+	+	-
8	+	+	+	-
9	-	-	-	+
10	+	-	-	+
11	-	+	-	+
12	+	+	-	+
13	-	-	+	+
14	+	-	+	+
15	-	+	+	+
16	+	+	+	+

Table 2: Matrix for screening of experiment.

Number of exp	k <sub>1</sub> (Y <sub>1</sub> )	k <sub>2</sub> (Y <sub>2</sub> )	α (Y <sub>3</sub> )	Rs (Y <sub>4</sub> )
1	1.03	1.17	1.13	0.96
2	0.75	0.85	1.14	0.94
3	1.03	1.17	1.14	0.93
4	0.68	0.77	1.13	0.93
5	1.03	1.18	1.15	0.96
6	0.73	0.84	1.16	0.94
7	1.03	1.18	1.15	0.96
8	0.70	0.80	1.14	0.94
9	1.03	1.17	1.13	0.98
10	0.75	0.85	1.14	0.94
11	1.03	1.17	1.14	0.93
12	0.68	0.77	1.13	0.93
13	1.03	1.18	1.15	0.95
14	0.73	0.84	1.16	0.94
15	1.03	1.18	1.15	0.97
16	0.70	0.80	1.14	0.95

Table 3: Results for outputs.

Number of exp	k <sub>1</sub>	k <sub>2</sub>	α	Rs
b <sub>1</sub>	-0.315	-0.36	0	-0.01625
b <sub>2</sub>	-0.025	-0.03	-0.005	-0.00875
b <sub>1</sub> b <sub>2</sub>	-0.025	-0.03	-0.01	0.00625
b <sub>3</sub>	0.00000	0.01000	0.01500	0.00875
b <sub>1</sub> b <sub>3</sub>	-4.16334E-17	-1.38778E-17	0	-0.00125
b <sub>2</sub> b <sub>3</sub>	0.01	0.01	-0.005	0.01625
b <sub>1</sub> b <sub>2</sub> b <sub>3</sub>	0.01	0.01	0	-0.00875
b <sub>4</sub>	0	5.55112E-17	-3.60822E-16	0.00375
b <sub>1</sub> b <sub>4</sub>	1.38778E-17	-6.93889E-17	0	-0.00125
b <sub>2</sub> b <sub>4</sub>	0	0	-2.77556E-17	0.00125
b <sub>1</sub> b <sub>2</sub> b <sub>4</sub>	0.01	0.01	0	-0.00875
b <sub>3</sub> b <sub>4</sub>	0	5.55112E-17	-2.77556E-17	-0.00125
b <sub>1</sub> b <sub>3</sub> b <sub>4</sub>	1.38778E-17	-1.38778E-17	0	0.00375
b <sub>2</sub> b <sub>3</sub> b <sub>4</sub>	0	0	2.77556E-17	0.00625
b <sub>1</sub> b <sub>2</sub> b <sub>3</sub> b <sub>4</sub>	0	-1.38778E-17	0	-0.00375
b <sub>0</sub>	0.8725	0.995	1.1425	0.946875

Table 4: Coefficients for mathematical models.

Number of exp.	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>
1	-	-	-
2	0	-	-
3	+	-	-
4	-	0	-
5	0	0	-
6	+	0	-
7	-	+	-
8	0	+	-
9	+	+	-
10	-	-	+
11	0	-	+
12	+	-	+
13	-	0	+
14	0	0	+
15	+	0	+
16	-	+	+
17	0	+	+
18	+	+	+
19	0	0	-
20	0	0	-
21	0	0	+
22	0	0	+

Table 5: Matrix of experiment for optimization.

metoprolol tartarate enantiomers. The results for analysis of variance concerning influence of acetonitrile content and β-CD content, as the most important inputs, are presented in Table 7 for output Y<sub>1</sub> and in Table 8 for output Y<sub>4</sub>.

Coefficients of determination (R<sup>2</sup>) and results for factor Fisher value (F) demonstrated good fitting of obtained results in mathematical model. Suitable three-D graphs are presented in Figure 1 for output Y<sub>1</sub> and in Figure 2 for output Y<sub>4</sub>.

Obtained three-D graphs gave information about influence of acetonitrile content and chiral modifcator concentration on metoprolol tartarate enantiomers separation. The connection between influence of the factors and outputs can be presented with second order polynoms. The obtained polynoms are presented as Equation 2 and Equation 3.

$$Y_1 = -3.585 + 0.059x_1 + 1.288x_2 - 0.004x_1^2 - 0.003x_1x_2 - 0.086x_2^2 \quad (\text{Eq. 2})$$

where is Y<sub>1</sub>-is retention factor of enantiomer R (+)

$$Y_4 = 6.918 - 0.52x_1 - 0.875x_2 + 0.022x_1^2 + 0.001x_1x_2 + 0.059x_2^2 \quad (\text{Eq. 3})$$

where is Y<sub>4</sub>-is resolution factor

As it could be seen from the Figure 1, retention factor of the first enantiomer has a higher value for higher content of β-CD and higher content of acetonitrile. Also, strong influence of acetonitrile content is obvious and drastical decrease of retention factor was observed. On the other hand, resolution factor is under strong influence of both investigated factors. It is clear that both factors must be carefully set in order to achieve acceptable separation of enantiomers investigated.

According to the presented results the best separation of metoprolol tartarate enantiomers can be achieved with mobile phase:

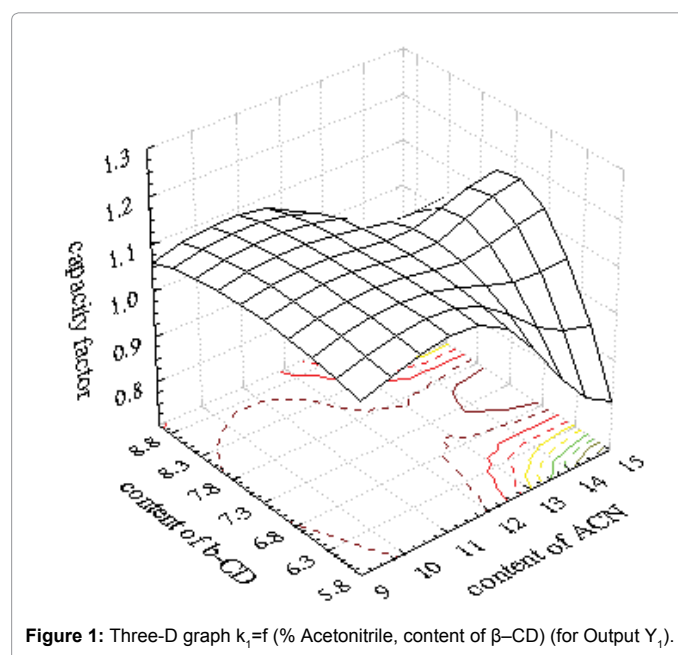


Figure 1: Three-D graph k<sub>1</sub>=f (% Acetonitrile, content of β-CD) (for Output Y<sub>1</sub>).

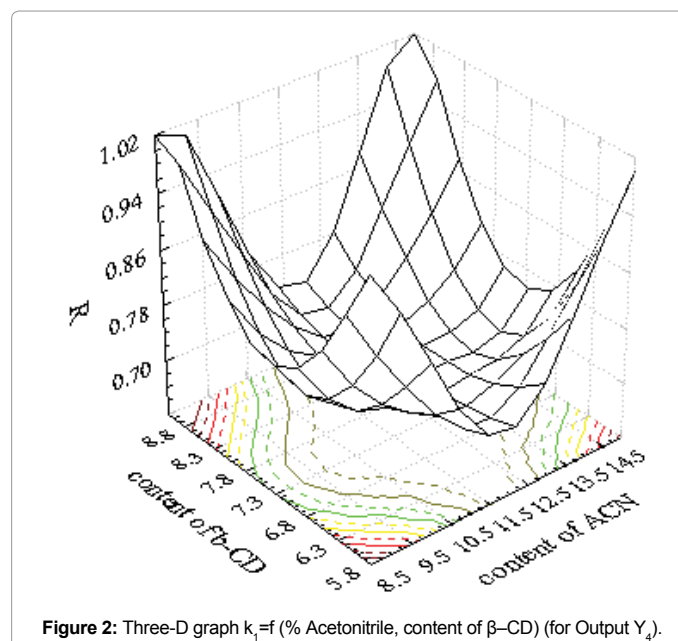


Figure 2: Three-D graph k<sub>1</sub>=f (% Acetonitrile, content of β-CD) (for Output Y<sub>4</sub>).

acetonitrile-5.87 mM  $\beta$ -CD (14.3:85.7), pH of the mobile phase adjusted to 3.0 and flow rate 1.5 mlmin<sup>-1</sup> at 35°C column temperature. Under these conditions the value of the resolution factor is 0.98. The representative chromatograms are presented in Figure 3.

## Conclusion

Applying experimental design it was possible to achieve optimal separation of metoprolol tartarate enantiomers performing a relatively small number of experiments. In this paper full factorial design 2<sup>4</sup> was used for experiment screening. Chromatographic behavior of investigated enantiomers was affected by acetonitrile content, chiral modifier content in the mobile phase and flow rate the most, which was demonstrated by obtained linear models. After experimental screening, RSM was used for optimization of RP-HPLC method and optimal chromatographic conditions were settled. The proposed methodology represents an efficient and easily attainable approach in resolving the problem of searching for optimal HPLC chromatographic conditions via experimental design.

Number of exp	k <sub>1</sub> (Y <sub>1</sub> )	k <sub>2</sub> (Y <sub>2</sub> )	$\alpha$ (Y <sub>3</sub> )	Rs (Y <sub>4</sub> )
1	1.03	1.17	1.13	0.96
2	1.05	1.18	1.12	0.68
3	0.75	0.85	1.14	0.94
4	1.31	1.48	1.13	0.72
5	1.05	1.19	1.13	0.67
6	1.14	1.28	1.12	0.68
7	1.03	1.17	1.14	0.93
8	1.05	1.18	1.13	0.67
9	0.68	0.77	1.13	0.93
10	1.03	1.18	1.15	0.96
11	1.1	1.23	1.12	0.67
12	0.73	0.84	1.16	0.94
13	1.32	1.51	1.14	0.72
14	1.09	1.23	1.13	0.67
15	1.15	1.31	1.14	0.67
16	1.03	1.18	1.15	0.96
17	1.03	1.17	1.14	0.67
18	0.7	0.8	1.14	0.94
19	1.04	1.2	1.13	0.66
20	1.05	1.19	1.13	0.67
21	1.1	1.22	1.13	0.67
22	1.09	1.23	1.13	0.66

Table 6: Results of experiment for optimization.

Source of variation	Sum of squares	d.f.	Mean square	F-ratio	F-tab
X <sub>1</sub>	0.21333333	1	0.21333333	22.15	F <sub>1,18</sub> =1.14
X <sub>2</sub>	0.00240833	1	0.00240833	0.25	
X <sub>1</sub> <sup>2</sup>	0.41423037	1	0.41423037	21.15	F <sub>3,16</sub> =3.16 F <sub>15,3</sub> =8.70
X <sub>2</sub> <sup>2</sup>	0.21781833	1	0.21781833	22.61	
Model	0.43356000	3	0.1445184	15.0029	
Error	0.173390	18	0.00963278		
Lack of fit	0.05539	15	0.00369	5.594	
Purely experimental uncertainty	0.01800	3	0.00066		
Total (corr)	0.59814	21			
R <sup>2</sup>			0.7143		

Table 7: Analysis of variance for variables and for the full regression for retention factor (k<sub>1</sub>).

Source of variation	Sum of squares	d.f.	Mean square	F-ratio	F-tab
X <sub>1</sub>	0.00187500	1	0.00187500	0.42	F <sub>1,17</sub> =1.45
X <sub>2</sub>	0.00020833	1	0.00020833	0.05	
X <sub>1</sub> <sup>2</sup>	0.20423045	1	0.20423045	46.19	F <sub>4,17</sub> =2.96 F <sub>14,3</sub> =8.71
X <sub>2</sub> <sup>2</sup>	0.08965974	1	0.08965974	20.28	
Model	0.29597352	4	0.0739934	16.7335	
Error	0.0751719	17	0.00442188		
Lack of fit	0.05171	14	0.004083	0.6805	
Purely experimental uncertainty	0.01800	3	0.00600		
Total (corr)	0.44086	21			
R <sup>2</sup>			0.7974		

Table 8: Analysis of variance for variables and for the full regression for resolution factor (Rs).

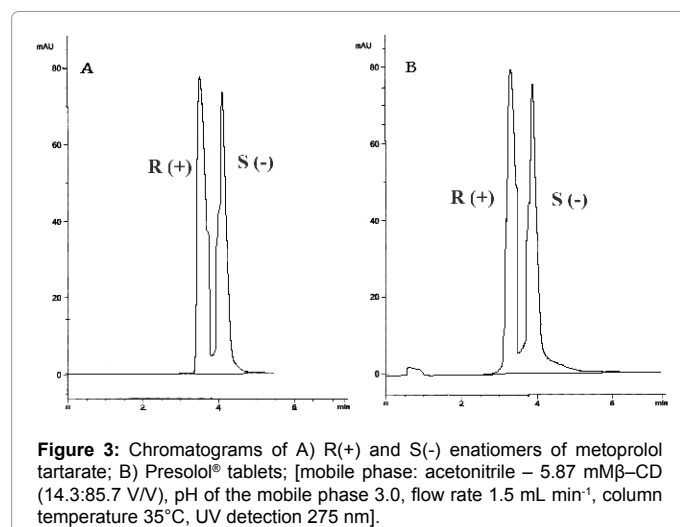


Figure 3: Chromatograms of A) R(+) and S(-) enantiomers of metoprolol tartarate; B) Presolol® tablets; [mobile phase: acetonitrile – 5.87 mM $\beta$ -CD (14.3:85.7 V/V), pH of the mobile phase 3.0, flow rate 1.5 mL min<sup>-1</sup>, column temperature 35°C, UV detection 275 nm].

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