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Analysis of metoprolol enantiomers via reverse phase (RP-HPLC) with M-ß-Cyclodextrin as mobile additive

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Analysis of metoprolol enantiomers via reverse phase (RP-HPLC) with M-ß-Cyclodextrin as mobile additive

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Abstract. Enantiomeric separation of the racemic metoprolol was investigated using a reverse phase HPLC (RP-HPLC) with Methyl-beta-cyclodextrin (M-B-CD) as chiral mobile phase additive. A comparison of the enantioseparation of the racemic metoprolol, the system suitability, linearity, accuracy, limit of detection and quantification was undertaken to show the performance between two C18 columns, a Zorbax Eclipse XDB C-18 column (15 cm x 10 mm, 10 µm) and a Syncronis C18 HPLC column (250 mm x 4.6 mm, 5.0 µm) using high performance liquid chromatography with the same condition of mobile phase composition, pH value of the mobile phase and concentration of chiral additives. The resolution was achieved using a mobile phase consisting of a mixture of aqueous solution (3.5 g M-ß-CD in 300 ml H_20), methanol with a volumetric ratio of 86:14 (v/v) and a flow rate of 1.0 ml/min and 0.5 ml/min for the columns respectively. Conversion of S-metoprolol found in this study are 60%, 51%, 15%.

Keywords: Metoprolol, S-metoprolol, RP-HPLC, chiral selector, enantioseparation, Methyl-B-cyclodextrin, lipase.

1. Introduction

Racemic metoprolol is also known as (+,-)1-(isopropylamino)-3-[p-(2-methoxyerhyl)phenoxy]-2-propanol and is a selective ß$_1$-blocker, which has an insignificant membrane-stabilising agent and does not display partial antagonist activity (Benfield, P., Clissold, S. P., & Brogden, 1986). It is used in the treatment of cardiovascular diseases (Grassi, 2018) though S-enantiomers have greater affinity (50 to 500 fold) for binding to the ß-adrenergic receptor than the R-enantiomer. The chiral stationary phase such as column OD (Agustian, Kamaruddin, & Aboul-Enein, 2017), column OJ (Banoth, Thakur, Bhaumik, & Banerjee, 2015) and column AD are used for the separation of racemic beta blockers. A commercially pure form chiral molecules are obtained in a common ways such as the resolution of racemates, chiral pool synthesis, laboratory invented chiral building, asymmetric synthesis and biotransformations, (Ettireddy, Chandupatla, & Veeresham, 2017). Recently, a new method has been developed by Younes

(2018) using methyl-beta Cyclodextrin as a chiral additive mobile phase for chiral separators via reverse phase high performance liquid chromatography (RP-HPLC). Cyclodextrin as a chiral cyclic oligosaccharide and specifically ß-cyclodextrin containing seven glucose units are used in chiral recognition techniques because their cavity can fit into a large number of molecules, available at relatively low cost.

2. Materials and Methods

2.1 Chemicals and reagents:

Racemic metoprolol, R-metoprolol, S-metoprolol, Methyl-beta-cyclodextrin, diethylamine, orthophosphoric acid, HPLC methanol, HPLC water, Acetonitrile, ethanol, Dimethylsulphoxide, dimethylformamide and lipase.

2.1.1 Analysis of enantioseparation of metoprolol

Instrument (A)

Chromatogram analysis was performed by an Agilent 1200 HPLC system constituted by an Agilent 1200 high performance auto sampler, a quartenary pump and a column oven Agilent 1200, an Agilent UV-VIS DAD detector and a chemstation data processing system. The mobile phase consisted of H_2O: Methanol: DEA (86:14:0.01) v/v/v using orthophosphoric acid adjusted until pH 2.7 with wavelength detection at 274 nm, flow rate 1.0 ml/min and injection volume 20 µl. The column used in the system was Ascentis C18 Supelco 10 µm particle size, L x I.D, 15 cm x 10 mm.

Instrument (B)

The analysis of racemic metoprolol enantiomers was performed using Shimadzu ultrafast liquid chromatography (UFLC) LC6AD which consisted of two units of LC6AD solvent delivery pump, one unit SIL 10AP auto sampler, a Photo Diode Array Detector, one unit CT-20AC column oven, one unit DGU-A3 degasser unit and a CBM-20A system controller with software using the Shimadzu LC solution Real Time application using a Syncronis C-18 column (150 mm, 4.6 mm, 0.5 µm). The condition used was (Aqueous solution: methanol: diethyl amine) (86 %:14 %:0.1 %) using orthophosphoric acid adjusted until pH 2.7 with wavelength detection at 274 nm, 0.5 ml/min of flow rate with 20 µl injection volume.

Sample preparation

Enantioseparation of metoprolol:

S-metoprolol and R-metoprolol was injected to identified the peaks based on the retention time. Racemic metoprolol of different concentrations was dissolved in solvent and filtered using syringe filter of a 0.45 µm prior to injection.

Lipase-catalyzed transesterification of metoprolol:

Enantioselective transesterification was chosen for he kinetic resolution of racemic metoprolol. Reaction was carried in a 150 ml Erlenmeyer flask. Substrate of racemic metoprolol was dissolved in 20 ml of solvent then adding the acyl agent and the enzyme that are shaken at 200 rpm and 45^{o}C in water bath shaker. Sample of 1 ml was taken at interval from the reaction medium for HPLC analysis.

3. Results

The chromatogram of the racemic metoprolol with mobile phase consisting of aqueous solution: methanol: diethylamine (86:14:0.1) v/v/v, the solution of M-ß-CD of 1.5 g in 1000 ml, 1.5 g in 500 ml and 3 g in 602 ml at pH 2.7 showed one peak at a retention time of 10.8 mins for 1.5 g M-ß-CD in 500 ml

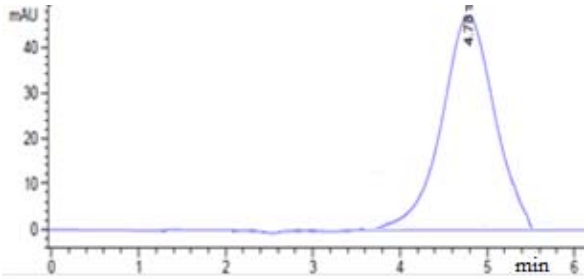

Figure 1: Chromatogram of racemic metoprolol (RS:4.781 mins), Mobile phase: aqueous solution/ methanol/diethylamine of (86:14:0.1) (v/v/v), 1. 5 g M-ß-CD in 500 ml, pH 2.7 (**A**).

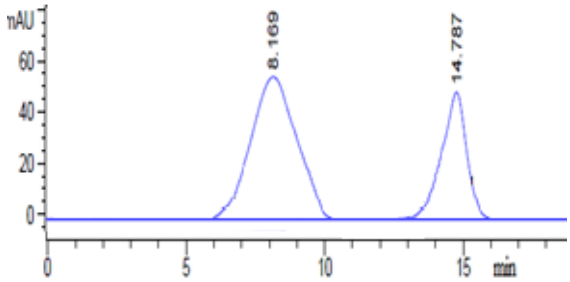

Figure 2: Chromatogram of enantioseparation racemic metoprolol (R:8.169 mins, S:14.787 mins), Mobile phase: aqueous solution/methanol/diethylamine of (86:14:0.1) (v/v/v), 3 g M-ß-CD in 500 ml, pH 2.7 (**A**).

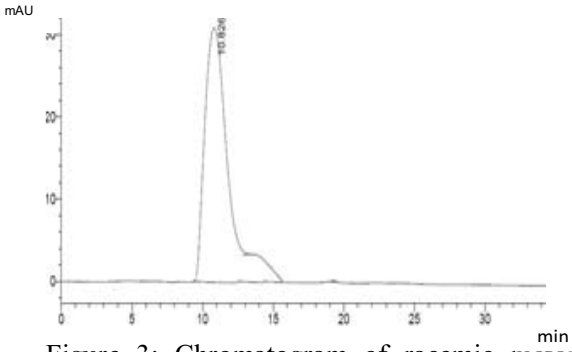

Figure 3: Chromatogram of racemic metoprolol (10.826 mins), Mobile phase: aqueous solution/ methanol/diethylamine of (86:14:0.1) (v/v/v), 1.5 g M-ß-CD in 500 ml, pH 2.7 (**B**).

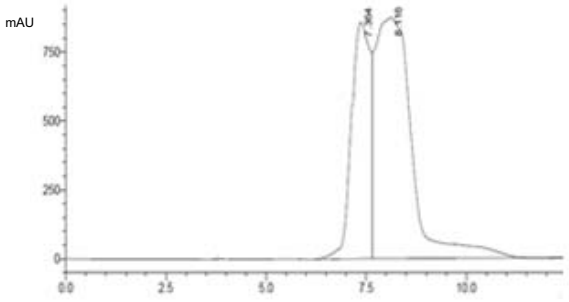

Figure 4: Chromatogram of enantioseparation of racemic metoprolol (R:7.364 mins, S:8.116 mins), Mobile phase: aqueous solution/methanol/diethylamine of (86:14:0.1) (v/v/v), 3.5 g M-ß-CD in 500 ml, pH 2.7 (**B**).

Table 1: Effect of M-B-CD on resolution (Ratio 86:14) (Aqueous solution: methanol)

Concentration M-B-CD	**A**	**B**
1.5 g in 1000 ml	No separation	No separation
1.5 g in 500 ml	No separation	No separation
3 g in 602 ml	7.9575	No separation
3/3.5 g in 300 ml	1.59555	0.104833

Table 2: Effect of solvents used to dilute metoprolol base (Ratio 86:14) (Aqueous: methanol)

M-B-CD in HPLC water	Solvent	Resolution (Rs)	
		Instrument A	Instrument B
3 g in 300 ml	Ethanol	0.1143	1.8811
3 g in 300 ml	Methanol	1.5955	-
3.5 g in 300 ml	Acetonitrile	-	0.104
3 g in 300 ml	Dimethylsulphoxide	-	No separation

3 g in 300 ml	Dimethylformamide	-	No separation
3 g in 602 ml	Acetonitrile	7.9575	-
3 g in 602 ml	Methanol	No separation	0.3333

Table 3: Effect of pH on the resolution using Column C-18 (Ratio 86:14) (Aqueous: methanol)

pH of mobile phase (M-B-CD)	Rs (Resolution)	
	Instrument **A**	Instrument **B**
2.01 (3 g in 602 ml)	7.9575	0.3333
1.80 (3.5 g in 300 ml)	1.5955	1.8811

Validation of Method:

The examination of linearity, system suitability, limit of detection (LOD) and limit of quantification (LOQ) was validated.

Suitability of system:

The equations used to calculate the retention factor, theoretical plate (N), resolution factor, separation factor and tailing factor of the enantiomer peaks according to Younes (2018) as listed below.

Table 4: System suitability (Ratio 86:14) (aqueous: methanol)

Solvent (metoprolol)	M-B-CD (g/ml)	Enantiomer	N	T	tR	K	a	Rs
Ethanol (**A**)	(3 g in 300 ml)	R enantiomer	266.93	0.37	14.78	1.19	2.09	3.89
		S enantiomer	5247.72	0.35	8.16	4.66		
Acetonitrile (**B**)	(3.5 g in 300 ml)	R enantiomer	31.89	0.46	7.05	0.55	1.24	0.10
		S enantiomer	18.60	0.11	7.68	0.69		

Linearity:

Three replicate of samples at different concentration levels of racemic metoprolol base was injected and formed a linear calibration curves of each enantiomer at the concentration range (Ratio 86: 14)(Aqueous: methanol).

Table 5: Regression equation and coefficient of each enantiomer

System	Enantiomer	Regression equation (Y)	Regression coefficient (R^2)
A	R	Y= 2147.8x + 6336.7	$R^2 = 0.9890$
	S	y = 1694.4x + 6052.8	$R^2 = 0.9987$
B	R	y = 35059x + 393573	$R^2 = 0.9995$
	S	y = 1694.4x + 6052.8	$R^2 = 0.9964$

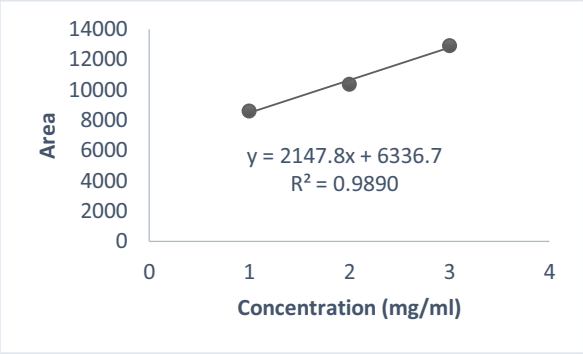

Figure 5: Calibration curve (**A**) of R-enantiomer of metoprolol base (3 g M-B-CD in 300 ml)

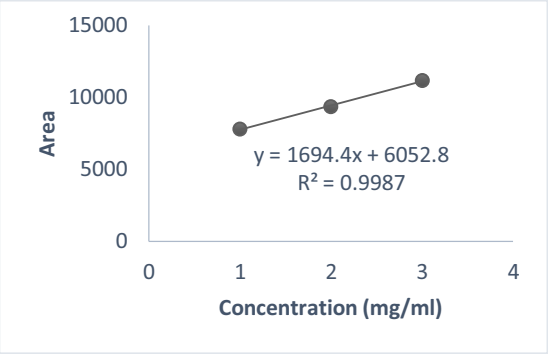

Figure 6: Calibration curve (**A**) of S-enantiomer of metoprolol base (3 g M-B-CD in 300 ml)

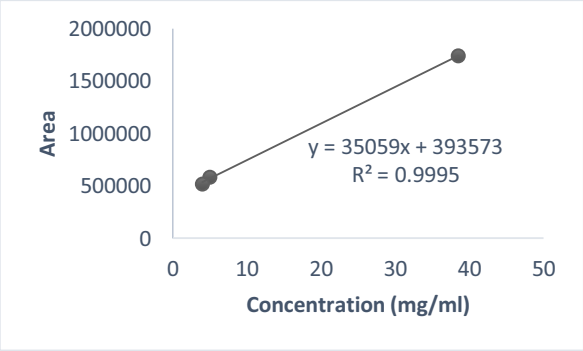

Figure 7: Calibration curve (**B**) of R-enantiomer of metoprolol base (3 g M-B-CD in 300 ml)

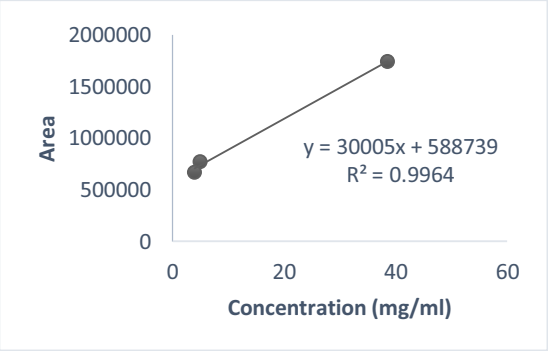

Figure 8: Calibration curve (**B**) of S-enantiomer of metoprolol base (3 g M-B-CD in 300 ml)

Table 6: Limit of detection (LOD) and Limit of quantification (LOQ) in mg/ml:

Instrument	LOD=3.3σ/S		LOD=10σ/S	
	R-metoprolol	S- metoprolol	R- metoprolol	S- metoprolol
A	6.7215	9.3879	20.3683	28.4482
B	58.2564	69.4761	176.5346	210.5339

3.1 Lipase-catalyzed transesterification of metoprolol

Lipase-catalyzed transesterification of racemic metoprolol was carried out. Racemic metoprolol (18.8mM) in organic solvent (20mL) was treated with vinyl acetate (67.68mM) in the presence of lipase (0.27mg/mL). The equation used to calculate the conversion of enantiomer, enantioselectivy (E) and enantiomeric excess of substrate (ee_s) and (ee_p) was adopted from Chen et al. (1982) and Long et al. (2005).

Table 7: Conversion of R-enantiomer and S-Enantiomer in a reaction condition (200rpm, 45^0C). RS-metoprolol (18.8Mm) in organic Solvent (20mL) was treated with vinyl acetate (67.68mM) In the presence of lipase (Candida Antartica Lipase) in 24 hours.

Lipase	X_s (%)	X_r (%)	X (%)	ee_S (%)	ee_P (%)	E-value
Candida antartica	15	69	44	37	16	2.98
Pseudomonas fluorescence	51	52	52	17	16	1.60
Mucor miehei	60	23	49	25	24	2.30

Conversion (X, total conversion; X_S, Conversion of S-metoprolol; X_R, Conversion of R-metoprolol; E, Enantioselectivity) [13-15] was calculated and the enantiomeric excess of substrate and product via HPLC analysis (Diacel Chiracel OD column, 80:20, hexane;2-propanol), 0.5ml/min flow rate, at 274nm.

4. Discussion

The ratio of the mobile phase followed the method developed by Younes (2018). The investigation of the enantioseparation was studied with different concentration of methyl-beta-cyclodextrin. It was found that all the enantioseparation was in 3/3.5 g of methyl-beta-cyclodextrin diluted in 300 ml of HPLC water. No separation was found in 1.5 g of 1000 ml, 1.5 g of 500 ml and 3 g of 600 ml of methyl-beta-cyclodextrin in HPLC water. The concentration required in this study was two times higher than the concentration used by Younes (2018) via conditioning the column with mobile phase prior to injections upon achieving the separation. The study then continued with screening the type of solvents used to dilute the racemic metoprolol powder. Separation of metoprolol enantiomer was found in ethanol and acetonitrile although there was no separation in methanol, DMSO or DMF. The effect of the mobile phase pH on the resolution of metoprolol enantiomers between C18 for instrument A and instrument B was 1.5955 and 1.8811 respectively. The analysis of lipase-catalyzed transesterification of metoprolol was performed. The reaction product was R-metoprolol ester and S-metoprolol. Enantiomeric excess of substrates increases as the time increase in a 5 hours' reaction and the concentration of S-metoprolol and R-metoprolol are shown in Table 7.

5. Conclusion

The enantioseparation of racemic metoprolol enantiomers was determined and performed on two C18 columns, a Zorbax Eclipse XDB C-18 column (15 cm x 10 mm, 10 µm) and a Syncronis C18 HPLC column (250 mm x 4.6 mm, 5.0 µm) using high performance liquid chromatography. The resolution was achieved using a mobile phase consisting of a mixture of aqueous solution (3.5 g M-ß-CD in 300 ml H20), a methanol with a volumetric ratio of 86:14 (v/v) and flow rate of 1.0 ml/min and 0.5 ml/min for the columns respectively. The chiral separation could aid to determine the enantiomers of the racemic metoprolol for the production of pure enantiomeric metoprolol in lipase-catalyzed reaction.

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References

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Agustian, J., Kamaruddin, A. H., & Aboul-Enein, H. Y. (2017). Factors screening to statistical experimental design of racemic atenolol kinetic resolution via transesterification reaction in organic solvent using free *Pseudomonas fluorescens* lipase. *Chirality*, *29*(7), 376–385. https://doi.org/10.1002/chir.22702

Banoth, L., Thakur, N. S., Bhaumik, J., & Banerjee, U. C. (2015). Biocatalytic Approach for the Synthesis of Enantiopure Acebutolol as a β1-Selective Blocker. *Chirality*, *27*(6), 382–91. https://doi.org/10.1002/chir.22444

Benfield, P., Clissold, S. P., & Brogden, R. N. (1986). Metoprolol. An update review of its pharmacodynamic and therapeutic efficacy, in hypertension, ischaemic heart disease, and related cardiovascular disorders. *Drugs*, *31*, 376–429.

Chen, C. S., Fujimoto, Y., Girdaukas, G., & Sih, C. J. (1982). Quantitative analyses of biochemical kinetic resolutions of enantiomers. *Journal of the American Chemical Society*, *104*(25), 7294–7299. https://doi.org/10.1021/ja00389a064

Ettireddy, S., Chandupatla, V., & Veeresham, C. (2017). Enantioselective Resolution of (R,S)-Carvedilol to (S)-(-)-Carvedilol by Biocatalysts. *Natural Products and Bioprospecting*, *7*(1), 171–179. https://doi.org/10.1007/s13659-016-0118-2

Grassi, G. (2018). Metoprolol in the treatment of cardiovascular disease: a critical reappraisal. https://doi.org/10.1080/03007995.2018.1479245org/10.1080/03007995.2018.1479245

Sing Long, W., Kamaruddin, A. H., & Bhatia, S. (2005). Enzyme kinetics of kinetic resolution of racemic ibuprofen ester using enzymatic membrane reactor. *Chemical Engineering Science*, *60*(18), 4957–4970. https://doi.org/10.1016/J.CES.2005.03.016

Younes, O. M., Ali, F. A., & Assaf, Z. Al. (2018). Enantioseparation of Metoprolol Tartrate using HPLC by Adding Methyl beta Cyclodextrin to the mobile Phase (As Chiral Additive). *Research Journal of Pharmacy and Technology*, *11*(9), 3937–3942. https://doi.org/10.5958/0974-360X.2018.00723.0

Agustian, J., Kamaruddin, A. H., & Aboul-Enein, H. Y. (2017). Factors screening to statistical experimental design of racemic atenolol kinetic resolution via transesterification reaction in organic solvent using free *Pseudomonas fluorescens* lipase. *Chirality*, *29*(7), 376–385. https://doi.org/10.1002/chir.22702

Banoth, L., Thakur, N. S., Bhaumik, J., & Banerjee, U. C. (2015). Biocatalytic Approach for the Synthesis of Enantiopure Acebutolol as a β1-Selective Blocker. *Chirality*, *27*(6), 382–91. https://doi.org/10.1002/chir.22444

Benfield, P., Clissold, S. P., & Brogden, R. N. (1986). Metoprolol. An update review of its pharmacodynamic and therapeutic efficacy, in hypertension, ischaemic heart disease, and related cardiovascular disorders. *Drugs*, *31*, 376–429.

Chen, C. S., Fujimoto, Y., Girdaukas, G., & Sih, C. J. (1982). Quantitative analyses of biochemical kinetic resolutions of enantiomers. *Journal of the American Chemical Society*, *104*(25), 7294–7299. https://doi.org/10.1021/ja00389a064

Ettireddy, S., Chandupatla, V., & Veeresham, C. (2017). Enantioselective Resolution of (R,S)-Carvedilol to (S)-(-)-Carvedilol by Biocatalysts. *Natural Products and Bioprospecting*, *7*(1), 171–179. https://doi.org/10.1007/s13659-016-0118-2

Grassi, G. (2018). Metoprolol in the treatment of cardiovascular disease: a critical reappraisal. https://doi.org/10.1080/03007995.2018.1479245org/10.1080/03007995.2018.1479245

Sing Long, W., Kamaruddin, A. H., & Bhatia, S. (2005). Enzyme kinetics of kinetic resolution of racemic ibuprofen ester using enzymatic membrane reactor. *Chemical Engineering Science*, *60*(18), 4957–4970. https://doi.org/10.1016/J.CES.2005.03.016

Younes, O. M., Ali, F. A., & Assaf, Z. Al. (2018). Enantioseparation of Metoprolol Tartrate using HPLC by Adding Methyl beta Cyclodextrin to the mobile Phase (As Chiral Additive). *Research Journal of Pharmacy and Technology*, *11*(9), 3937–3942. https://doi.org/10.5958/0974-360X.2018.00723.0

Agustian, J., Kamaruddin, A. H., & Aboul-Enein, H. Y. (2017). Factors screening to statistical

experimental design of racemic atenolol kinetic resolution via transesterification reaction in organic solvent using free *Pseudomonas fluorescens* lipase. *Chirality*, *29*(7), 376–385. https://doi.org/10.1002/chir.22702

Banoth, L., Thakur, N. S., Bhaumik, J., & Banerjee, U. C. (2015). Biocatalytic Approach for the Synthesis of Enantiopure Acebutolol as a β1-Selective Blocker. *Chirality*, *27*(6), 382–91. https://doi.org/10.1002/chir.22444

Benfield, P., Clissold, S. P., & Brogden, R. N. (1986). Metoprolol. An update review of its pharmacodynamic and therapeutic efficacy, in hypertension, ischaemic heart disease, and related cardiovascular disorders. *Drugs*, *31*, 376–429.

Chen, C. S., Fujimoto, Y., Girdaukas, G., & Sih, C. J. (1982). Quantitative analyses of biochemical kinetic resolutions of enantiomers. *Journal of the American Chemical Society*, *104*(25), 7294–7299. https://doi.org/10.1021/ja00389a064

Ettireddy, S., Chandupatla, V., & Veeresham, C. (2017). Enantioselective Resolution of (R,S)-Carvedilol to (S)-(-)-Carvedilol by Biocatalysts. *Natural Products and Bioprospecting*, *7*(1), 171–179. https://doi.org/10.1007/s13659-016-0118-2

Grassi, G. (2018). Metoprolol in the treatment of cardiovascular disease: a critical reappraisal. https://doi.org/10.1080/03007995.2018.1479245org/10.1080/03007995.2018.1479245

Sing Long, W., Kamaruddin, A. H., & Bhatia, S. (2005). Enzyme kinetics of kinetic resolution of racemic ibuprofen ester using enzymatic membrane reactor. *Chemical Engineering Science*, *60*(18), 4957–4970. https://doi.org/10.1016/J.CES.2005.03.016

Younes, O. M., Ali, F. A., & Assaf, Z. Al. (2018). Enantioseparation of Metoprolol Tartrate using HPLC by Adding Methyl beta Cyclodextrin to the mobile Phase (As Chiral Additive). *Research Journal of Pharmacy and Technology*, *11*(9), 3937–3942. https://doi.org/10.5958/0974-360X.2018.00723.0