

## Product Information



Metoprolol  
Propranolol  
Chiral Separation

Enantioselective determination of  
 $\beta$ -blockers in human plasma and saliva

Date: 02.01.2017  
Author: Anna Bergmann

**From application and phase screening  
to two publications**



**A success story with CHIRAL ART**

## Product Information



Metoprolol  
Propranolol  
Chiral Separation

### Enantioselective determination of $\beta$ -blockers in human plasma and saliva

Date: 02.01.2017

Author: Anna Bergmann

This product information sums up the success story of CHIRAL ART from an existing application and chiral phase screening to two publications describing the LC-MS method for enantioselective determination of  $\beta$ -blockers in complex matrices.

With CHIRAL ART Cellulose-SB, the following aims are achieved:

- **enantioselective determination of propranolol and metoprolol**
- **baseline separation with high resolution of the two enantiomers**
- **determination in complex matrices: human plasma and saliva**

As the results are presented in two official publications, the outstanding performance of CHIRAL ART has been confirmed by different scientists with an independent point of view of the chiral YMC columns!

## Contents

1 Introduction .....	3
2 YMC application for propranolol .....	4
3 Analysis of propranolol enantiomers in plasma samples .....	5
4 Phase screening and method development for metoprolol .....	7
5 Analysis of metoprolol in plasma and saliva samples .....	8
6 Summary .....	10
7 Literature .....	10

## 1 Introduction

The **existing YMC application** (V140131A3) for **propranolol** with **CHIRAL ART Cellulose-SB** was modified by the working group of M. Abdel-Rehim for their field of application and validated afterwards. The **first paper** was published in October 2015.

In summer 2015, they requested a **chiral phase screening** for the enantioselective separation of **metoprolol**. The result was that (R)- and (S)-metoprolol are well separated with CHIRAL ART Cellulose-SB, too. They successfully transferred the existing method for propranolol to metoprolol. With this data, they have published **a second article** for the determination of metoprolol in plasma and saliva in August 2016.

On the following pages, the **success story** with CHIRAL ART from phase screening, performed by YMC Europe GmbH in Dinslaken, to a published method for the **enantioselective determination** of the two  $\beta$ -blockers metoprolol and propranolol with CHIRAL ART Cellulose-SB in **complex sample matrices** like human plasma and saliva is presented.

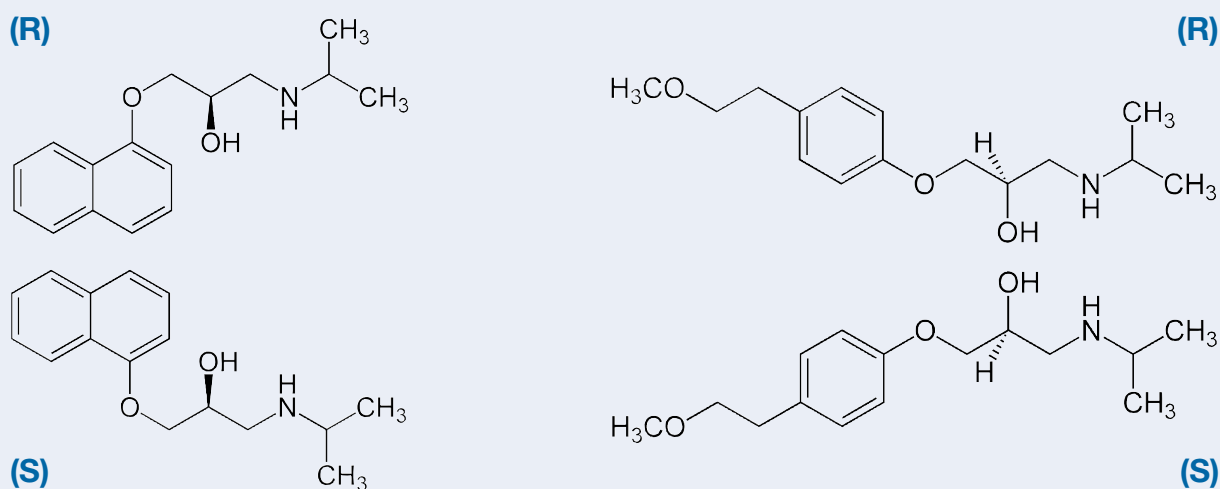


Figure 1: Structures of the two enantiomers of propranolol (left) and metoprolol (right).

## Product Information



Metoprolol  
Propranolol  
Chiral Separation

Enantioselective determination of  
 $\beta$ -blockers in human plasma and saliva

Date: 02.01.2017  
Author: Anna Bergmann

## 2 YMC application for propranolol

Based on an existing application (V140131A3) for propranolol with CHIRAL ART Cellulose-SB (Figure 2), the working group of M. Abdel-Rehim started to develop the LC-MS method for enantioselective determination of propranolol.

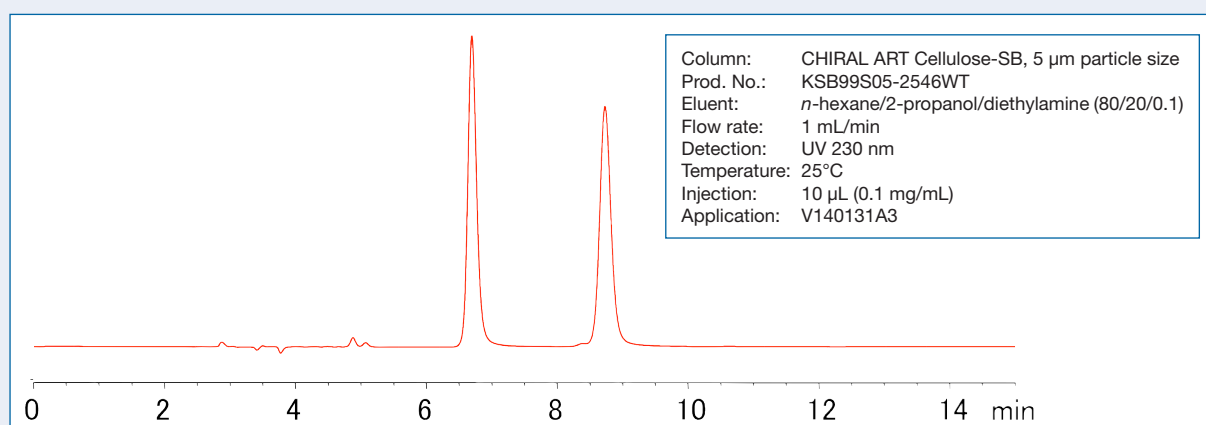


Figure 2: Enantioselective determination of propranolol using CHIRAL ART Cellulose-SB.

Taking this application as a basis, H. Elmongy et al. used CHIRAL ART Cellulose-SB for LC-MS separation and determination of propranolol enantiomers in human plasma samples – a very complex matrix. The results were summarised in the article “Online post-column solvent assisted and direct solvent-assisted electrospray ionization for chiral analysis of propranolol enantiomers in plasma samples” [1] and are presented in the following chapter.

Metoprolol  
Propranolol  
Chiral Separation

**Enantioselective determination of  
 $\beta$ -blockers in human plasma and saliva**

Date: 02.01.2017  
Author: Anna Bergmann

## 3 Analysis of propranolol enantiomers in plasma samples

The paper "Online post-column solvent assisted and direct solvent-assisted electrospray ionization for chiral analysis of propranolol enantiomers in plasma samples" published in October 2015 in the Journal of Chromatography A [1] summarises the results for method development and validation made by the working group of M. Abdel-Rehim for the separation of propranolol enantiomers in a very complex and difficult matrix: human plasma.

### Analytical conditions

Table 1: Analytical conditions for the separation of propranolol enantiomers [1]

Column	CHIRAL ART Cellulose-SB, 5 $\mu$ m particle size, 150 x 4.6 mm ID
Prod. No.	KSB99S05-1546WT
Mobile phase	<i>n</i> -hexane/isopropanol (80/20) with 0.1% ammonium hydroxide
Makeup solvent	0.5% formic acid in isopropanol
Flow rate	0.8 mL/min
Injection volume	50 $\mu$ L
Detection	ESI-MS
Sample preparation	Micro-extraction of both enantiomers by packed C18 sorbent (MEPS)

### Usage of makeup solvents

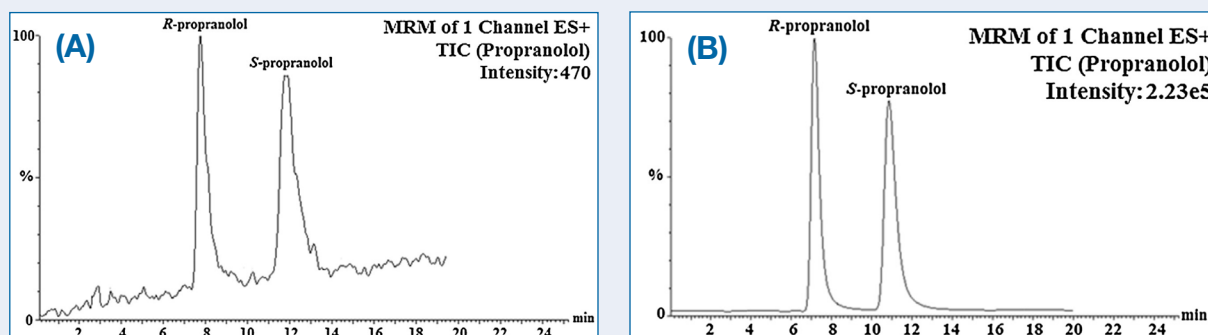


Figure 3: Enantioselective separation of propranolol standard solution A) without and B) with makeup solvent [1].

Figure 3 shows the importance of using a makeup solvent to improve the chromatographic result with MS detection. The use of makeup solvent provides:

- **Stable baseline**
- **Improved peak shape**
- **Improved resolution**

## Product Information



Metoprolol  
Propranolol  
Chiral Separation

### Enantioselective determination of $\beta$ -blockers in human plasma and saliva

Date: 02.01.2017  
Author: Anna Bergmann

#### Method transfer to plasma samples

After development with propranolol standard solutions, the method was successfully transferred to human plasma samples (Figure 4). Even in this complex matrix very low limits of quantification and detection can be achieved (Table 2).

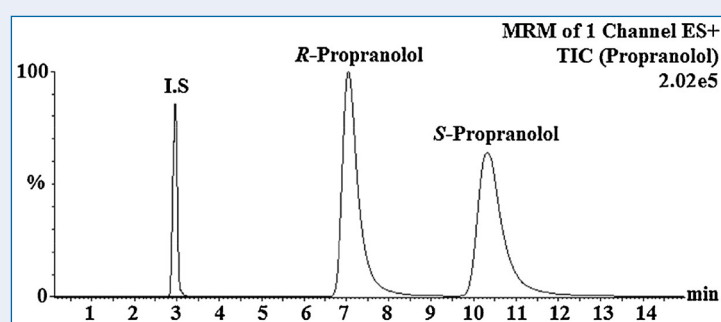


Figure 4: Enantioselective separation of propranolol in human plasma samples [1].

Table 2: LOD and LOQ for propranolol enantiomers in human plasma

Limit of detection (LOD)	10 ng/mL in human plasma
Limit of quantitation (LOQ)	50 ng/mL in human plasma

## 4 Phase screening and method development for metoprolol

After the successful implementation of the LC-MS method for enantioselective determination of the  $\beta$ -blocker propranolol in human plasma, M. Abdel-Rehim and his working group requested a chiral phase screening for another  $\beta$ -blocker: metoprolol.

As a separation in RP-mode with MS detection was the goal, immobilised chiral YMC phases were screened. The separation of the two enantiomers of this drug was tested on three immobilised chiral YMC phases: CHIRAL ART Amylose-SA, CHIRAL ART Cellulose-SB and CHIRAL ART Cellulose-SC.

In order to improve resolution and retention, the composition of the mobile phase was varied. The results showed that the best separation of (R)- and (S)-metoprolol is achieved using a CHIRAL ART Cellulose-SB column.

### Metoprolol: Best separation on CHIRAL ART Cellulose-SB

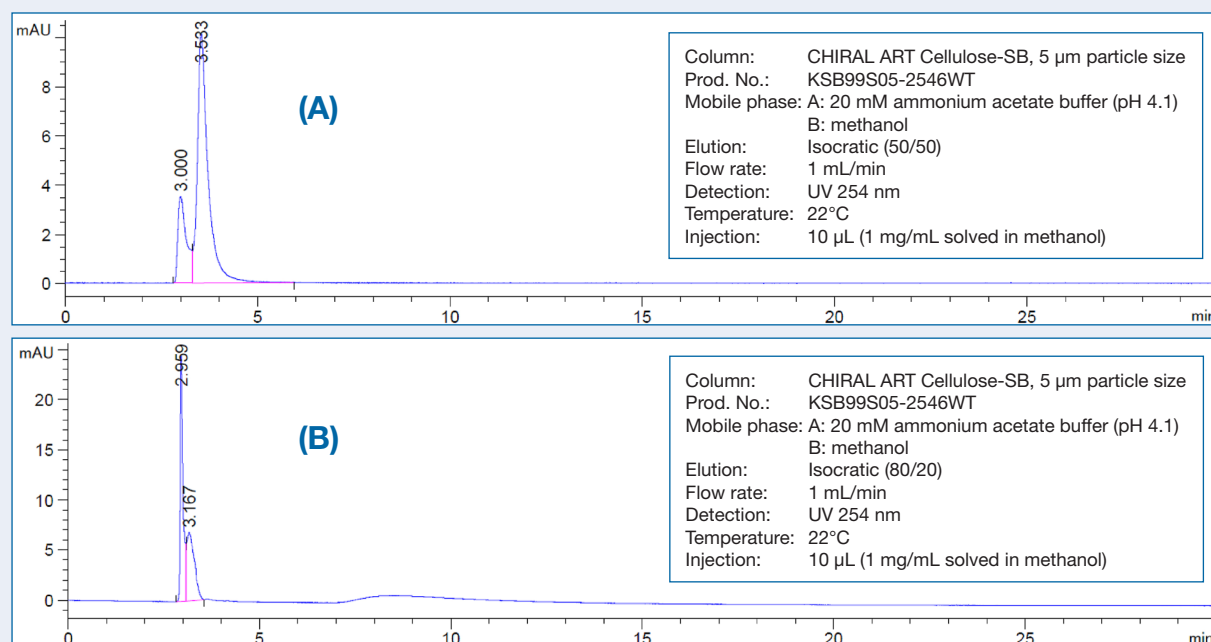


Figure 5: Determination of metoprolol with CHIRAL ART Cellulose-SB: mobile phase composition (A) 50/50 and (B) 80/20.

With the data from the phase screening showing that CHIRAL ART Cellulose-SB was the best choice, H. Elmongy et al. successfully transferred the already existing LC-MS method for propranolol determination to the separation of metoprolol. In addition to the enantioselective determination of  $\beta$ -blockers in human plasma, they expanded the application range to another complex matrix: human saliva. The results are published in the journal Biomedical Chromatography [2] in August 2016. The paper is summarised in the following chapter.



Metoprolol  
Propranolol  
Chiral Separation

Enantioselective determination of  
 $\beta$ -blockers in human plasma and saliva

Date: 02.01.2017  
Author: Anna Bergmann

## 5 Analysis of metoprolol in plasma and saliva samples

Based on the results of the first published paper and the phase screening, M. Abdel-Rehim and his working group developed a method for the enantioselective determination of metoprolol in human plasma and saliva samples. The paper "Determination of metoprolol enantiomers in human plasma and saliva samples utilizing micro-extraction by packed sorbent and liquid chromatography-tandem mass spectrometry" published in August 2016 in the journal Biomedical Chromatography [2] highlights the results of this study.

Table 3: Analytical conditions for the separation of metoprolol enantiomers [2]

Column	CHIRAL ART Cellulose-SB, 5 $\mu$ m particle size, 150 x 4.6 mm ID
Prod. No.	KSB99S05-1546WT
Mobile phase	<i>n</i> -hexane/isopropanol (80/20) with 0.1% ammonium hydroxide
Makeup solvent	0.5% formic acid in isopropanol
Flow rate	0.8 mL/min
Injection volume	50 $\mu$ L
Detection	ESI-MS
Sample preparation	Micro-extraction of both enantiomers by packed C18 sorbent (MEPS)

### Usage of makeup solvents

The use of makeup solvents for metoprolol analysis also improves the chromatographic results with MS detection regarding stability of baseline, resolution and peak shape (Figure 6).

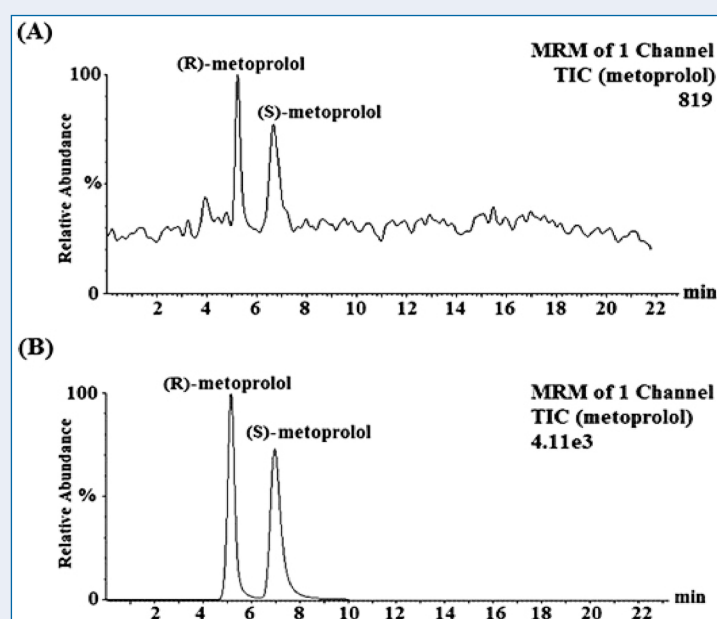


Figure 6: Enantioselective separation of metoprolol in plasma (A) without and (B) with makeup solvent [2].



## Product Information

Metoprolol  
Propranolol  
Chiral Separation

### Enantioselective determination of $\beta$ -blockers in human plasma and saliva

Date: 02.01.2017  
Author: Anna Bergmann

As with propranolol, the method transfer from standards to the samples of interest – human plasma and saliva – was successfully achieved (Figure 7). As the method can determine the therapeutic level of metoprolol in human plasma and saliva, it can be used in clinical laboratories for therapeutic drug monitoring. Again, very low limits of quantitation and detection can be achieved for both matrices, plasma and saliva (Table 4).

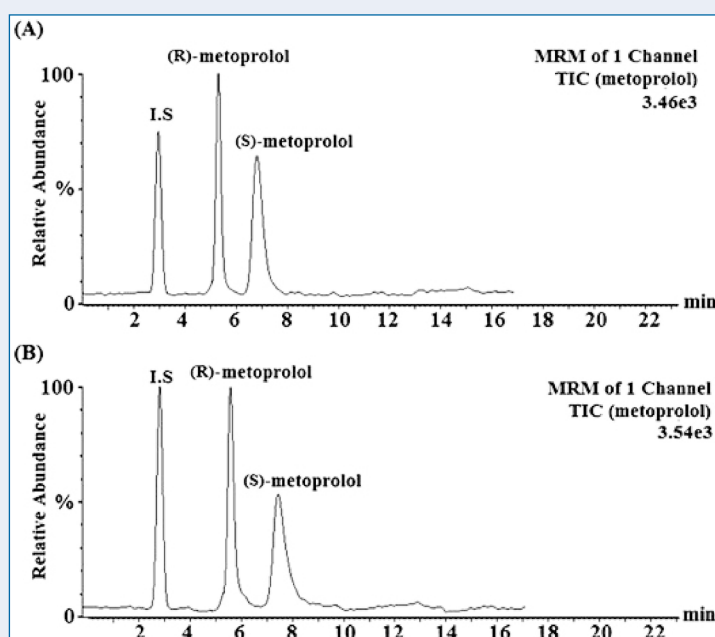


Figure 7: Enantioselective separation of (R)- and (S)-metoprolol in human (A) plasma and (B) saliva samples [2].

Table 4: LOD and LOQ for metoprolol enantiomers in human plasma and saliva

Limit of detection (LOD)	0.5 ng/mL in human plasma and saliva
Limit of quantitation (LOQ)	1.5 ng/mL in human plasma and saliva

## 6 Summary

YMC provides:

- **coated and immobilised chiral phases**
- **support for chiral separations:**  
**from applications to phase screenings and method development**
- **full reproducible applications**

With CHIRAL ART Cellulose-SB, the following aims are achievable:

- **enantioselective determination of  $\beta$ -blockers**
- **baseline separation with high resolution**
- **determination in complex matrices**

## 7 Literature

[1] H. Elmongy, et al., *Online post-column solvent assisted and direct solvent assisted electrospray ionization for chiral analysis of propranolol enantiomers in plasma samples*, J. Chromatogr. A (2015).

[2] H. Elmongy, et al., *Determination of metoprolol enantiomers in human plasma and saliva samples utilizing microextraction by packed sorbent and liquid chromatography-tandem mass spectrometry*, Biomed. Chromatogr. (2016).