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Chiral separation of beta-blockers by high-performance liquid chromatography and determination of bisoprolol enantiomers in surface waters

Marijana Pocrnić[1], Martin Ansorge[2], Magda Dovhunová[2], Iva Habinovec[1], Eva Tesařová[2], and Nives Galić[1]

[1] *Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia*
[2] *Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Prague, Czech Republic*

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Beta-blockers are chiral compounds with enantiomers that have different bioactivity, which means that while one is active, the other can be inactive or even harmful. Due to their high consumption and incomplete degradation in waste water, they may reach surface waters and affect aquatic organisms. To address this issue we developed a chromatographic method suitable for determining beta-blocker enantiomers in surface waters. It was tested on five beta-blockers (acebutolol, atenolol, bisoprolol, labetalol and metoprolol) and validated on bisoprolol enantiomers. Good enantioseparation of all analysed beta-blockers was achieved on the Chirobiotic V column with the mobile phase composed of methanol/acetic acid/triethylamine (100/0.20/0.15 v/v/v) at a flow rate of 0.5 mL/min and column temperature of 45 °C. Method proved to be linear in the concentration range from 0.075 μg/mL to 5 μg/mL, and showed good recovery. The limits of bisoprolol enantiomer detection were 0.025 μg/mL and 0.026 μg/mL and of quantification 0.075 μg/mL and 0.075 μg/mL. Despite its limitations, it seems to be a promising method for bisoprolol enantiomer analysis in surface water samples. Further research could focus on waste water analysis, where enantiomer concentrations may be high. Furthermore, transferring the method to a more sensitive one such as liquid chromatography coupled with tandem mass spectrometry and using ammonium acetate as the mobile phase additive instead of acetic acid and triethylamine would perhaps yield much lower limits of detection and quantification.

KEY WORDS: acebutolol; atenolol; Chirobiotic V column; Croatia; Czech Republic; enantioseparation; HPLC; labetalol; metoprolol; water analysis

Beta-adrenergic blocking agents, more commonly known as beta-blockers, are cardiovascular drugs used for the treatment of hypertension and coronary artery diseases such as angina pectoris and acute myocardial infarction (1). These compounds are chiral, with one or more stereogenic centres. As with many other chiral pharmaceuticals, one enantiomer can be bioactive while the other can be inactive or even toxic. For example, (S)-propranolol is 100 times more potent than (R)-propranolol. In addition, since (R)-propranolol can inhibit the conversion of thyroxin to triiodothyronine, it can be used to treat hyperthyroidism in patients with bradycardia, asthma, or where the use of racemate is contraindicated due to the adrenergic effect of (S)-propranolol (2).

Since beta-blockers are among the most common pharmaceuticals, high levels of the unchanged compound and their metabolites may end up in waste waters through excretion. Although waste waters are processed in waste water treatment plants, some pollutants are not completely removed, and beta-blockers may reach surface waters (3). In the last decade, beta-blockers have received an increasing attention as emerging pollutants, as exposure to higher levels can affect the growth, reproduction, heart rate, and cause stress response in some fish and mollusc species (4).

Currently, risk assessments of chiral pharmaceuticals are limited to racemates, even though individual enantiomers can have different effects on aquatic organisms. Furthermore, human metabolism or waste water treatment can convert one enantiomer into its counterpart (chiral inversion) and their 1:1 ratio may change in favour of one or another in surface waters. This is why future risk assessments of pharmaceuticals should extend their scope to individual enantiomers.

There are but a few studies reporting on enantiospecific toxicity of beta-blockers. Stanley et al. (5) reported three times greater toxicity of (S)-propranolol on *Pimephales promelas* growth rate than the (R)-enantiomer. In contrast, de Andrés et al. (6) reported four times greater toxicity of (R)-atenolol on *Tetrahymena thermophila* (6). Sun et al. (7) could not find enantiospecific toxicity of propranolol and metoprolol to embryos and larvae of *Danio rerio* but did

Corresponding author: Marijana Pocrnić, Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102 a, 10000 Zagreb, Croatia, E-mail: *mpocrnic@chem.pmf.hr*

observe changes in gene transcriptions that might indicate enantioselectivity.

Separation of enantiomers in beta-blockers has already been studied to some extent. Methods that are used are liquid chromatography (LC) with ultraviolet–visible spectrophotometry (UV/VIS) or mass spectrometry (MS) and capillary electrophoresis. The most common are direct LC methods with various chiral stationary phases based on polysaccharides (8–11), cyclodextrins (12), or macrocyclic antibiotics (13–16). Enantiomers can be separated in normal or reverse phase modes with various solvents such as hexane or acetonitrile and methanol, respectively. Various additives such as triethylamine, diethylamine, or acetic acid, can also be added to improve the resolution of enantiomers (8–16).

There are only a few papers describing the analysis of enantiomers of beta blockers in waste and surface waters. Water samples are purified and pre-concentrated by solid phase extraction using different kinds of cartridges (11, 17–18).

Since bisoprolol is the most commonly used beta-blocker in Croatia and Czech Republic (19, 20), its occurrence in surface and waste waters in these countries is quite likely. Methods used so far for bisoprolol determination in waste and river waters included LC coupled with triple quadrupole (21–22) and gas chromatography (GC) coupled with MS (23), but none involved chiral separation and could not provide information about single enantiomer content. Methods that have been used for chiral separation have only been validated for determining bisoprolol content in pharmaceutical products (9, 16).

Here we present a chromatographic method we developed for chiral separation of five beta-blockers (acebutolol, atenolol, bisoprolol, labetalol, and metoprolol) and its validation for bisoprolol enantiomer determination in surface water samples taken in Croatia and the Czech Republic.

MATERIALS AND METHODS

Chemicals

Standards of (±)-atenolol, (±)-acebutolol, (±)-labetalol and (±)-bisoprolol were purchased from Sigma-Aldrich (St. Louis, MO, USA) and (±)-metoprolol tartrate was purchased from Tokyo Chemical Industry (Tokyo, Japan). All standards were of high-purity grade (>98 %). High-purity grade (99 %) triethylamine (TEA) was purchased from Tokyo Chemical Industry, and glacial acetic acid (HAc) from Alkaloid AD Skopje (Skopje, North Macedonia). HPLC-purity grade methanol (MeOH) was purchased from J. T. Baker (Gliwice, Poland). Sodium hydroxide was of p.a. grade and purchased from Kemika (Zagreb, Croatia). Ultrapure water was obtained from a Milli-Q Advantage A10 purification system (Merck, Kenilworth, NJ, USA).

Standard solutions

Stock solutions of standards were prepared by weighing the appropriate amount of each beta-blocker and dissolving it in methanol to give the final concentration of 1 mg/mL. The method was optimised with standard solutions prepared by diluting stock solution with methanol to achieve the final concentration of 100 µg/mL. For method validation and determination of bisoprolol in surface waters, standard solutions were prepared by diluting the stock solution with methanol to achieve desired concentrations.

Sample preparation

Water samples were taken from the Sava River in Zagreb, Croatia and from the Kunraticky stream (Kunratice) and a nearby biological pond in Prague, Czech Republic. The stream runs near a hospital and receives wastewater discharged from its treatment system. Samples were collected upstream and downstream of the discharge location and were filtered through 12 µm pore size filter paper and stored at 4 °C until analysis.

Samples were prepared for analysis following a modified method by Meierjohann et al. (21). Before preparation, we adjusted the pH of water samples and of ultrapure water for solid phase extraction (SPE) to 10 with a 1 mol/L solution of sodium hydroxide. For solid phase extraction we used the Oasis HLB cartridges (6 mL, 500 mg) (Waters Corporation, Milford, MA, USA). Cartridges were preconditioned with methanol and ultrapure water. A sample volume of 250 mL was loaded on the cartridges at a flow rate of about 10 mL/min. After washing the cartridges with ultrapure water and drying them with vacuum, beta-blockers were eluted with methanol (6 mL). Aliquots were transferred to vials and analysed with a high-performance liquid chromatograph (HPLC).

Method development, instrumentation, and chromatographic conditions

We used an Agilent 1220 Infinity series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump, an autosampler, a thermostated column compartment, and a variable wavelength detector.

To develop the best HPLC method for chiral separation of beta-blockers acebutolol (ACE), atenolol (ATE), metoprolol (MET), labetalol (LAB) and bisoprolol (BIS) we evaluated four columns [Chiralpak IA and Chiralpak IC (Chiral Technologies Europe, Strasbourg, France) and Chirobiotic T and Chirobiotic V (Advanced Separation Technologies Inc., Whippany, NJ, USA)] with different mobile phases (consisting of *n*-hexane and methanol or ethanol) and different mobile phase additives (triethylamine, diethylamine, acetic acid, or isopropylamine). Seeing that the best results in terms of separation of all enantiomers were achieved on the Chirobiotic V column using polar ionic mode, where the mobile phase consisted of methanol and small amounts of acid and base, we then also evaluated

different ratios of acetic acid and triethylamine or diethylamine and the effects of column temperature and flow rate on enantioseparation for all of the analysed beta-blockers. This narrowed down the choice to the Chirobiotic V column (5 µm thick, 250×4.6 mm), mobile phase consisting of methanol plus acetic acid and triethylamine in the ratio 100:0.20:0.15 (v/v/v), flow rate of 0.5 mL/min, and column temperature of 45 °C. Detection was performed at 230 nm. The injection volume was 10 µL.

Method validation

The method was validated for determination of bisoprolol enantiomers. Enantiomeric fractions were calculated using equation [1]:

$$EF = \frac{E1}{E1 + E2} \times 100\ \% \qquad [1]$$

where E1 and E2 were the peak areas of the first and second eluting enantiomers of bisoprolol, respectively.

Linearity was tested on six standard solutions in the concentration range from 0.075 µg/mL to 5 µg/mL. Linear regression was tested by plotting peak areas against these six concentrations.

Instrumental limits of detection (IDL) were determined based on the calibration curve, as described in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (24), from equation [2]:

$$IDL = \frac{3.3\ \sigma}{S} \qquad [2]$$

where σ was residual standard deviation of a regression line, and S the slope estimated from the calibration curve in the concentration range from 0.025 µg/mL to 0.25 µg/mL. The calculated IDL was confirmed by analysing standard solutions of bisoprolol enantiomers prepared at IDL concentrations. After injecting a standard solution five times, IDL was confirmed if the signal-to-noise ratio (*S/N*) was above 3 every time. Instrumental limits of quantification (IQL) were obtained with the *S/N* ratio method by preparing and injecting standard solution five times. The suggested concentration was confirmed as IQL if the *S/N* ratio was above 10 every time.

RESULTS

The best results in terms of enantiomer separation were achieved with the Chirobiotic V column and mobile phase consisting of methanol/acetic acid/triethylamine in ratio 100:0.20:0.15 (*v*/*v*/*v*) with flow rate of 0.5 mL/min and column temperature of 45 °C. Table 1 shows the retention factor (k), resolution (R_s), and selectivity factor (α) for each analyte. The retention times of all analytes were within acceptable limits and lasted from 10 to 25 min. Baseline enantiomer separation was achieved with all beta-blockers but labetalol (having two stereogenic centres, i.e. four enantiomers), whose first two enantiomers were only partially separated (Figure 2).

We also tried analysing a mixture of enantiomers of all four beta blockers. The results were good for all analytes but the metoprolol and bisoprolol enantiomers, as one bisoprolol enantiomer co-eluted with one metoprolol enantiomer. We then tried different ratios of acetic acid and triethylamine and different flow rates and column temperatures but none improved the separation in the mixture.

Because of this reason and because bisoprolol is the most common beta-blocker in Croatia and Czech Republic and is the most likely to contaminate surface waters in these countries, we proceeded with method validation for bisoprolol alone.

As the standard solution we used was a racemic mixture, we could not identify the elution order of (R)- and (S)-enantiomers, so we named the first eluted enantiomer BIS1 and the second BIS2. Enantiomeric fractions of BIS1 and

Table 1 Chromatographic parameters for beta-blockers separated on the Chirobiotic V column using the MeOH:HAc:TEA (100:0.2:0.15) mobile phase, flow rate of 0.5 mL/min, and column temperature of 45 °C (detection wavelength 230 nm)

Analyte	Enantiomer	Retention time/min	R_s	k	α
Acebutolol	E1	13.19	1.43	1.12	1.12
	E2	13.99		1.24	
Atenolol	E1	14.53	1.37	1.33	1.10
	E2	15.38		1.46	
Bisoprolol	E1	10.85	1.30	0.74	1.12
	E2	11.43		0.83	
Labetalol	E1	17.49	0.65	1.77	1.03
	E2	18.11	3.06	1.82	1.26
	E3	21.16		2.30	
	E4	23.67	2.41	2.69	1.17
Metoprolol	E1	11.43	1.30	0.84	1.12
	E2	12.04		0.93	

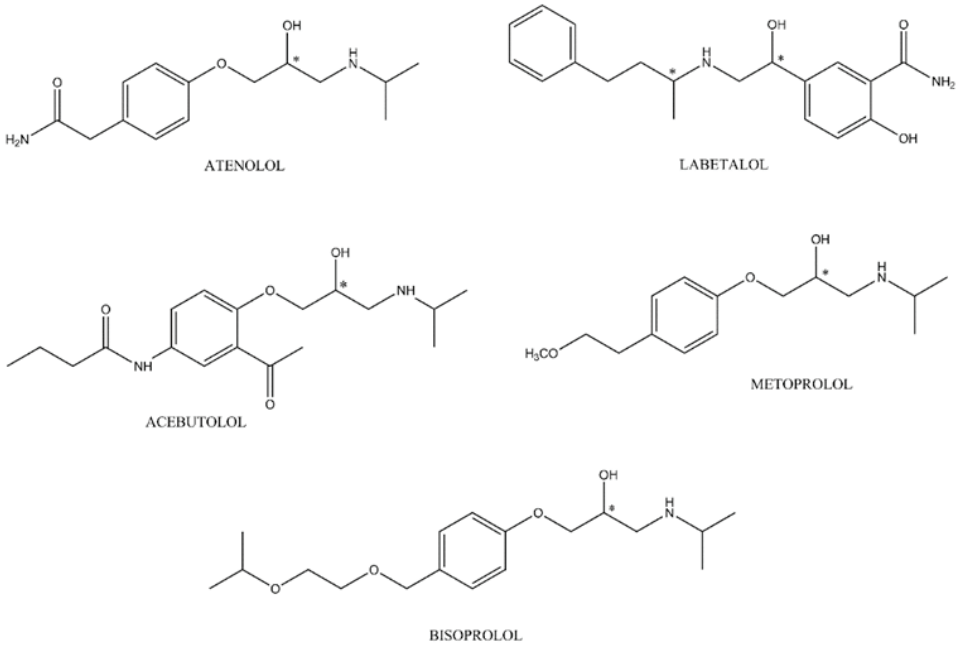

Figure 1 Structures of the analysed beta-blockers (chiral centres are marked with an asterisk)

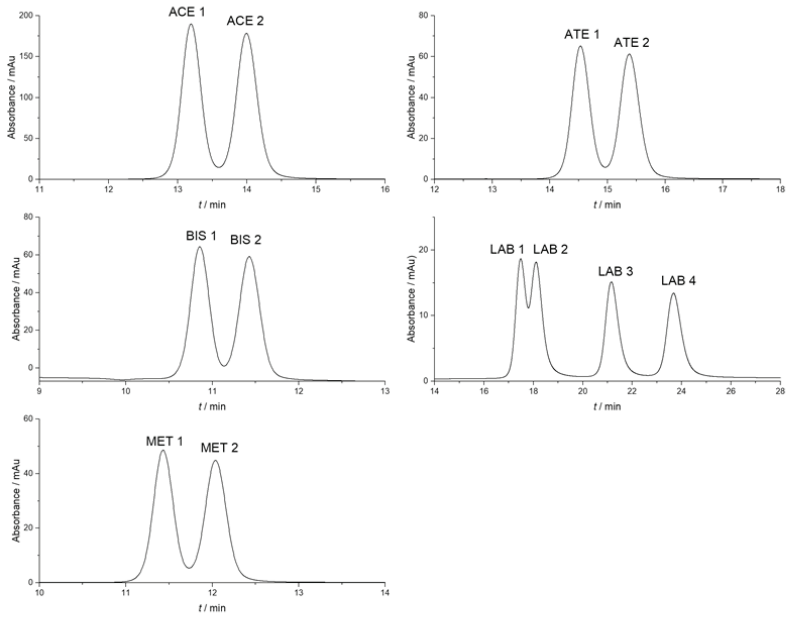

Figure 2 Chromatographic separation of acebutolol, atenolol, bisoprolol, labetalol, and metoprolol enantiomers on the Chirobiotic V column using the MeOH:HAc:TEA (100:0.2:0.15) mobile phase, flow rate of 0.5 mL/min, and column temperature of 45 °C (detection wavelength 230 nm)

BIS2 were 49.7 % and 50.3 %, respectively, confirming the racemic nature of the standard solution.

The IDL of each bisoprolol enantiomer, determined by linear regression, was 0.025 µg/mL for BIS1 and 0.026 µg/mL for BIS2. These limits were confirmed by injecting standard solution at calculated limit levels (0.025 µg/mL) with *S/N* ratios higher than 3. The IQL (*S/N* ratio >10) was 0.075 µg/mL for both enantiomers. These concentrations equal 0.6 µg/L and 1.8 µg/L in surface waters, respectively. Good linearity was achieved for both bisoprolol enantiomers in the concentration range of 0.075–5 µg/mL, with correlation coefficients above 0.998. This range equals 1.8–120 µg/L of each bisoprolol enantiomer in surface waters.

To determine the applicability of this method for bisoprolol enantiomer analysis in real samples, we analysed

Table 2 Expected and calculated concentrations of bisoprolol enantiomers and their recoveries in spiked samples of distilled and surface waters from Zagreb and Prague

	Expected γ (μg/L)		Calculated γ (μg/L)		Recovery (%)	
	BIS 1	BIS 2	BIS 1	BIS 2	BIS 1	BIS 2
Distilled water 1	12.00	12.00	12.02	11.85	100.2	98.8
Distilled water 2	240.00	240.00	265.71	264.58	110.7	110.2
Prague 1	12.00	12.00	13.18	13.19	109.8	109.9
Prague 2	9.36	9.36	10.08	10.08	107.7	107.7
Prague 3	8.16	8.16	9.44	9.49	115.7	116.3
Sava 1	1.80	1.80	2.27	2.35	126.3	130.3
Sava 2	6.00	6.00	8.14	8.30	135.7	138.3
Sava 3	12.00	12.00	13.90	14.31	115.8	119.3

the solid-phase extracts of surface water samples (taken in Prague and Zagreb) for bisoprolol content. Before solid-phase extraction, however, we added known amounts of standard bisoprolol solution to samples of distilled and surface waters to determine the rate of elution of the analytes from cartridges. These samples were prepared in the same way as regular samples. The recoveries of bisoprolol enantiomers in spiked distilled water at the two concentrations (99–111 %) showed good extraction of bisoprolol.

Samples from the Sava were spiked at three concentrations, 1.80 μg/L (Sava 1), 6.00 μg/L (Sava 2), and 12.00 μg/L (Sava 3). Samples from the Kunraticky stream (Prague 1 and Prague 3) and the nearby pond (Prague 2) were spiked at 12.00 μg/L or lower, according to Table 2. (Figure 3). The recoveries ranged from 100 % to 136 % for enantiomer BIS1 and from 99 % to 138 % for enantiomer BIS2, which is an acceptable range (Table 2). Comparing the recoveries of different samples spiked with 12.00 μg/L of bisoprolol, we can conclude that this method of sample preparation yielded good repeatability, with relative standard deviation of <5 % for both enantiomers.

We also tested the method on surface water samples without spiking them with standard solutions but found no bisoprolol enantiomers. This finding will be addressed further in discussion.

DISCUSSION AND CONCLUSIONS

Although the method developed in this study showed better enantiomer separation for each beta-blocker alone than reported previously (1, 9), it failed in separating enantiomers in the mixture of metoprolol and bisoprolol

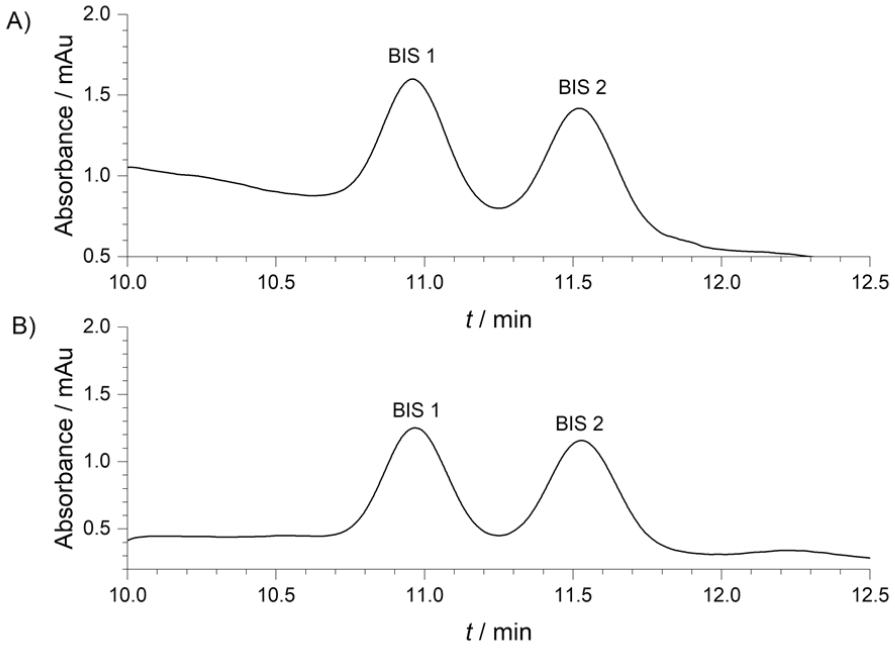

Figure 3 Chromatograms of samples from the Kunraticky stream (A) and the Sava River (B), spiked with bisoprolol enantiomers at 12 μg/L after solid-phase extraction

and should perhaps be limited to analysing bisoprolol alone, especially as its consumption and the associated risk of surface water contamination is the highest, at least in Croatia and the Czech Republic. Furthermore, the consumption of metoprolol is not very high in these countries, especially not in Croatia, and its occurrence in surface waters is unlikely, so it is not expected to interfere with bisoprolol enantiomer analysis in surface waters.

Our failure to find bisoprolol enantiomers in unspiked (and therefore real) surface water samples either suggests that bisoprolol is successfully removed in waste water treatment plants or points to another limitation of our method, which is that its detection limits are too high. Further analysis of waste water before and after treatment could give a better insight into the levels of bisoprolol occurring in these countries.

Even with these limitations, however, our method has shown adequate recoveries with spiked samples and could be used to determine bisoprolol enantiomers in surface waters, which, to our knowledge, has not been reported so far. Future research could therefore focus on bisoprolol enantiomer content in waste water collected by treatment plants, where its concentrations may be high. Furthermore, transferring the method to a more sensitive one – such as liquid chromatography coupled with tandem mass spectrometry and using ammonium acetate as the mobile phase additive instead of acetic acid and triethylamine – would perhaps yield much lower limits of detection and quantification (25).

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Conflict of interests

None to declare.

REFERENCES

1. Poirier L, Tobe SW. Contemporary use of β-blockers: clinical relevance of subclassification. Can J Cardiol 2014;30(Suppl 5):S9–15. doi: 10.1016/j.cjca.2013.12.001
2. Nguyen LA, He H, Pham-Huy C. Chiral drugs: an overview. Int J Biomed Sci 2006;2:85–100. PMID: 23674971
3. Hernando MD, Gómez MJ, Agüera A, Fernández-Alba AR. LC-MS analysis of basic pharmaceuticals (beta-blockers and anti-ulcer agents) in wastewater and surface water. Trend Anal Chem 2007;26:581–94. doi: 10.1016/j.trac.2007.03.005
4. Massarsky A, Trudeau VL, Moon TW. β-Blockers as endocrine disruptors: the potential effects of human β-blockers on aquatic organisms. J Exp Zool 2011;315:251–65. doi: 10.1002/jez.672
5. Stanley JK, Ramirez AJ, Mottaleb M, Chambliss CK, Brooks BW. Enantiospecific toxicity of the β-blocker propranolol to *Daphnia magna* and *Pimephales promelas*. Environ Toxicol Chem 2006;25:1780–6. doi: 10.1897/05-298r1.1
6. de Andrés F, Castañeda G, Ríos Á. Use of toxicity assays for enantiomeric discrimination of pharmaceutical substances. Chirality 2009;21:751–9. doi: 10.1002/chir.20675
7. Sun L, Xin L, Peng Z, Jin R, Jin Y, Qian H, Fu Z. Toxicity and enantiospecific diffrences of two β-blockers, propranolol and metoprolol, in the embryos and larvae of Zebrafish (*Danio rerio*). Environ Toxicol 2014;29:1367–78. doi: 10.1002/tox.21867
8. Stringham RW, Ye YK. Chiral separation of amines by high-performance liquid chromatography using polysaccharide stationary phases and acidic additives. J Chromatogr A 2006;1101:86–93. doi: 10.1016/j.chroma.2005.09.065
9. Ali I, Gaitonde VD, Aboul-Enein HY, Hussain A. Chiral separation of β-adrenergic blockers on CelluCoat column by HPLC. Talanta 2009;78:458–63. doi: 10.1016/j.talanta.2008.11.043
10. Santoro MIRM, Cho HS, Kedor-Hackmann ERM. Enantiomeric separation and quantitative determination of atenolol in tablets by chiral high-performance liquid chromatography. Drug Dev Ind Pharm 2000;26:1107–10. doi: 10.1081/DDC-100100275
11. Morante-Zarcero S, Sierra I. Comparative HPLC methods for β-blockers separation using different types of chiral stationary phases in normal phase and polar organic phase elution modes. Analysis of propranolol enantiomers in natural waters. J Pharm Biomed Anal 2012;62:33–41. doi: 10.1016/j.jpba.2011.12.029
12. Armstrong DW, Chen S, Chang C, Chang S. A new approach for the direct resolution of racemic beta adrenergic blocking agents by HPLC. J Liq Chromatogr 1992;15:545–56. doi: 10.1080/10826079208017191
13. Bosáková Z, Cuřínová E, Tesařová E. Comparison of vancomycin-based stationary phases with different chiral selector coverage for enantioselective separation of selected drugs in high-performance liquid chromatography. J Chromatogr A 2005;1088:94–103. doi: 10.1016/j.chroma.2005.01.017
14. Mistry B, Leslie JL, Eddington ND. Enantiomeric separation of metoprolol and α-hydroxymetoprolol by liquid chromatography and fluorescence detection using a chiral stationary phase. J Chromatogr B 2001;758:153–161. doi: 10.1016/S0378-4347(01)00166-9
15. Evans SE, Davies P, Lubben A, Kasprzyk-Hordern B. Determination of chiral pharmaceuticals and illicit drugs in wastewater and sludge using microwave assisted extraction, solid-phase extraction and chiral liquid chromatography coupled with tandem mass spectrometry. Anal Chim Acta 2015;882:112–26. doi: 10.1016/j.aca.2015.03.039
16. Hefnawy MM, Sultan MA, Al-Shehri MM. Development of an HPLC method for the quantitation of bisoprolol enantiomers in pharmaceutical products using a teicoplanin chiral stationary phase and fluorescence detection. J Liq Chromatogr R T 2006;29:2901–14. doi: 10.1080/10826070600978302
17. Bagnall JP, Evans SE, Wort MT, Luben AT, Kasprzyk-Hordern B. Using chiral liquid chromatography quadrupole time-of-flight mass spectrometry for the analysis of pharmaceuticals and illicit drugs in surface and wastewater

at the enantiomeric level. J Chromatogr A 2012;1249:115–29. doi: 10.1016/j.chroma.2012.06.012
18. Barclay VKH, Tyrefors NL, Johansson IM, Pettersson CE. Chiral analysis of metoprolol and two of its metabolites, α-hydroxymetoprolol and deaminated metoprolol, in wastewater using liquid chromatography-tandem mass spectrometry. J Chromatogr A 2012;1269:208–17. doi: 10.1016/j.chroma.2012.09.090
19. Agency for Medicinal Products and Medical Devices of Croatia (HALMED). Annual report on drug utilization for 2017 [displayed 17 October 2018]. Available at http://www.halmed.hr
20. State Institute for Drug Control. Annual summary on drug deliveries for 2017 [displayed 18 December 2018]. Available at http://www.sukl.cz
21. Meierjohann A, Brozinski JM, Kronberg L. Seasonal variation of pharmaceutical concentrations in a river/lake system in Eastern Finland. Enivoron Sci Processes Impacts 2016;18:342–9. doi: 10.1039/C5EM00505A
22. Lee HB, Sarafin K, Peart TE. Determination of β-blockers and β_2-agonists in sewage by solid-phase extraction and liquid chromatography-tandem mass spectrometry. J Chromatogr A 2007;1148:158–67. doi: 10.1016/j.chroma.2007.03.024
23. Miège C, Favier M, Brosse C, Canler JP, Coquery M. Occurrence of betablockers in effluents of wastewater treatment plants from the Lyon area (France) and risk assessment for the downstream rivers. Talanta 2006;70:739–44. doi: 10.1016/j.talanta.2006.07.002
24. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonized Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1) [displayed 27 November 2018]. Available at https://database.ich.org/sites/default/files/Q2_R1__Guideline.pdf.
25. Beesley TE, Lee JT. Method development strategy and applications update for CHIROBIOTIC chiral stationary phases. J Liq Chromatogr R T 2009;32:1733–67. doi: 10.1080/10826070902959489

Kiralna separacija beta-blokatora tekućinskom kromatografijom visoke djelotvornosti i određivanje enantiomera bisoprolola u površinskim vodama

Beta-blokatori su kiralni spojevi s enantiomerima različite bioaktivnosti, dakle, dok je jedan enantiomer aktivan, drugi može biti neaktivan ili čak štetan za organizam. Zbog njihove visoke potrošnje i nepotpune razgradnje u pogonima za preradu otpadnih voda, postoji mogućnost da se pojave u prirodnim vodama i negativno utječu na vodene organizme. Stoga je u ovom radu razvijena kromatografska metoda za određivanje enantiomera beta-blokatora u prirodnim vodama. Metoda je testirana na pet beta-blokatora (acebutolol, atenolol, bisoprolol, labetalol i metoprolol) te validirana za enantiomere bisoprolola. Dobra enantioseparacija svih analiziranih beta-blokatora postignuta je na koloni Chirobiotic V sastava mobilne faze metanol/octena kiselina/trietilamin (100:0,2:0,15 *v/v/v*) pri protoku od 0,5 mL/min i temperaturi od 45 °C. Metodom je postignuta dobra linearnost u području od 0,075 µg/mL do 5 µg/mL s dobrim analitičkim povratom. Granice detekcije pojedinih enantiomera bisoprolola bile su 0,025 µg/mL i 0,026 µg/mL, a granice kvantifikacije 0,075 µg/mL za oba enantiomera. Unatoč ograničenjima metode, pokazala se kao obećavajuća metoda analize enantiomera bisoprolola u površinskim vodama. Daljnja istraživanja mogla bi se izvoditi na otpadnim vodama, gdje bi koncentracije enantiomera mogle biti više. Također, korištenjem osjetljivije metode, primjerice vezanoga sustava tekućinske kromatografije i tandemne spektrometrije masa, te korištenjem amonijeva acetata kao aditiva mobilnoj fazi umjesto octene kiseline i trietilamina, mogle bi se postići znatno niže granice detekcije i kvantifikacije.

KLJUČNE RIJEČI: acebutolol; analiza vode; atenolol; Chirobiotic V kolona; Češka; enantioseparacija; HPLC; Hrvatska; labetalol; metoprolol