

Quantitative Characterization of Derivatization Effects in Separations Techniques

I. Derivatization of Ibuprofen

Anca-Gabriela Cârje¹, Zoltán Juvancz², Béla Tőkés¹

¹University of Medicine and Pharmacy, Tirgu Mureş, Physical Chemistry Laboratory, Romania

²Department of Environmental Engineering, Óbuda University, Budapest, Hungary, juvancz.zoltan@rkk.uni-obuda.hu

Abstract: Quantitative relations were studied between chiral separation parameters and chemical forms of ibuprofen. The structure, partition coefficients, enthalpy, entropy and free enthalpy change of inclusion complex formations of enantiomers have been established. Results of different methods were compared. The authors emphasis distinguished study of chiral recognition and the derivatization procedures.

Keywords: chiral separation methods; ibuprofen; derivatization; structure – selectivity relationship

1 Introduction

In this paper the authors perform a study of chemical structure dependence of separation methods' parameters among different conditions. Special attention was paid to effects of derivatization processes of analytes for the chiral recognition of permethylated β -cyclodextrin.

The chiral or asymmetric molecules cannot superimpose with molecules which are their mirror images [1]. A chiral molecule and its mirror image isomer are together called an enantiomeric pair. The members of enantiomer pairs are distinguished with (*R*) and (*S*) prefix according to Chan-Ingold-Prelog rules [2]. An old assignment of enantiomer pairs are the (+) and (-) symbols. Negative and positive symbols show the direction of torsion of polarized light (486 nm) in the solution of given enantiomer.

Enantiomeric pairs can show rather different biological effects, in spite of their very similar structures [3, 4]. The Contergan Scandal was a tragic example for the different effects of enantiomers. The Contergan (Thalodimide) a sedative pill was

sold as mixtures of members of enantiomers. The (*R*) isomer was harmless, but the (*S*) caused serious birth defects [5]. The possible biological difference between members of enantiomeric pairs has forced the authorities to introduce the directives of enantiomeric pure medicines [6]. These directives expect to commercialize only one member of an enantiomeric pair. A product can contain less than 0.1% of the other members of the enantiomeric pair. The requirement of chiral pure medicines makes the chiral selective analyses necessary.

Enantiomeric separations or chiral selective separations are a very challenging branch of chromatography [7]. The members of enantiomeric pairs are indistinguishable in homogeneous space. Their separations require a chiral separation agent: chiral stationary phase (CPS) or chiral mobile phase additives (CMA). The chiral separations need simultaneous three point interactions between the selectors and selectands [8]. It is not enough to have appropriate types of interaction groups in selector and selectand, but their appropriate steric arrangements are also required for the three point interactions. The separation of a given enantiomeric pair needs “tailor made” separation agents, which has its three interaction groups in appropriate steric arrangements. Trial and error method are used in several occasions to find the appropriate separation agent for the required chiral separation.

An efficient chromatographic system can separate enantiomeric pairs having only 0.1 kJ/mol interaction energy difference toward to the chiral separation media [9].

The chiral separations can be improved to choose appropriate derivatives of enantiomeric pairs and selectors [10, 11].

Our aim is to flash the theoretical background of successful chiral separations. This paper shows that the acidic hydrogen of acids can improve their chiral recognition via hydrogen bonds comparing the methyl ester derivatives of the these acids. Using our results may help to find the appropriate combinations of selector-selectand pairs for chiral separations in a more effective way than only the application of trial and error method.

Ibuprofen was chosen as model compound and starting material for further studies. Ibuprofen is an important nonsteroidal anti-Inflammatory drug (NSAID) used in the treatment of pain and inflammation in a variety of musculoskeletal and rheumatic disorders [12]. Ibuprofen, like other 2-arylpropionate derivatives (including ketoprofen, flurbiprofen, naproxen, etc.), does contain an asymmetric stereo center in the α -position of the propionate moiety. As such, there are two possible enantiomers of ibuprofen, with the potential for different biological effects and metabolism for each enantiomer [13, 14]. Indeed, the (*S*)-(+)-ibuprofen was found to be the active form both *in vitro* and *in vivo*. It was logical, then, that there was the potential for improving the selectivity and potency of ibuprofen formulations by marketing ibuprofen as a single-enantiomer product (dexibuprofen) [15]. Further *in vivo* testing, however, revealed the existence of an

isomerase (*alpha-methylacyl-CoA racemase*), which converted (*R*-)-ibuprofen to the active (*S*+)-enantiomer [16].

Enantiospecific analytical methodology is suitable for the determination of the enantiomeric ratio of the drug and its metabolites. The determination of enantiomeric ratio is essential in order to evaluate the significance of stereoselectivity both in terms of drug action and disposition. Enantiomer selective GC [17], HPLC [18], SFC [19] and CE [20] methods have been developed for the ibuprofen. Many of these methods use cyclodextrins as chiral selective agents. In this study, the stereo selective separation mechanism of ibuprofen has been studied using permethylated- β -cyclodextrin as chiral selective agent. The sterical arrangements and thermodynamic parameters of interactions between the permethylated- β -cyclodextrin and enantiomers of ibuprofens have been established. The methodology of our report is same as the one used in our previous studies [21, 22]. It was established, that ibuprofen and its methyl ester derivatives have different chiral recognition mechanism among GC conditions. These results were compared and interpreted with the literature data. Our data was compared to literature data of CE separations referring to the selectivity differences between undissociated and the dissociated forms of ibuprofens toward to β -cyclodextrin.

2 Experimental

2.1 Compounds

The (*R,S*)-Ibuprofen or (*R,S*)-2-(4-(2methylpropyl)-phenyl)-propionic acid, Nitroso(N)-methyl-carbamide methylation agent, n-hexane and diethyl ether are product of Sigma-Aldrich.

2.2 Instrumental

The samples were measured on Shimadzu QP5000 GC/MS instrument (Shimadzu Inc., Kyoto, Japan). The experimental conditions were the following: column, 25 m x 0.22 mm FSOT; stationary phase, Cydex-B (0.25 μ m); (SGE); carrier, He. The used chiral selective stationary phase (Cydex-B) is a mixture of silicone polymer and permethylated- β -cyclodextrin.

2.3 Procedures

Methyl ester of ibuprofen was synthesized by standard methylation procedure with Nitroso(N)-methyl-carbamide via diazomethane intermediate [23]. The retention times of free acid and methyl esters of ibuprofen were measured at least 4 times in different temperatures. This data was sufficient for thermodynamic calculations.

3 Results

The structural formulas of enantiomers of ibuprofen (*RS*)-2-(4-(2methylpropyl)-phenyl)-propionic acid are presented in Figure 1.

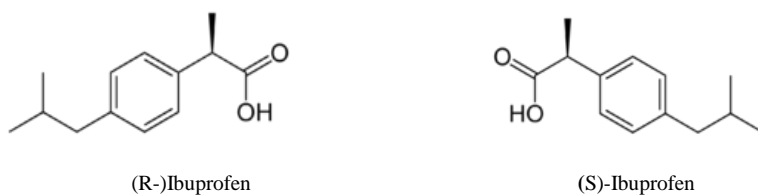


Figure 1

Structures of ibuprofen enantiomers. Figures show how the enantiomers of ibuprofen are mirror images of each other, but are not superimposable.

Figure 2 is a representative chromatogram of ibuprofen enantiomeric pairs in free acid forms among GC conditions.

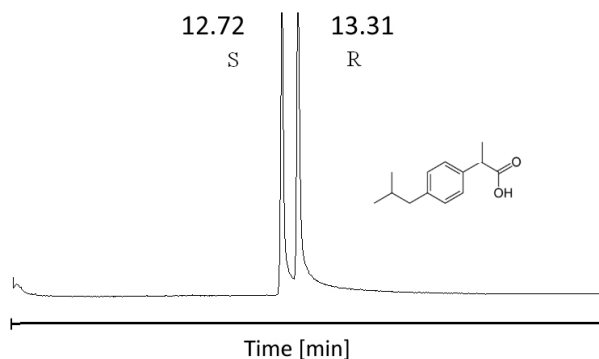


Figure 2

The separation of ibuprofen enantiomers in acid form with GC.

Parameters: Instrument, Shimadzu QP5000 GC/MS; column, 25 m x 0.22 mm FSOT; stationary phase, Cydex-B (0.25 μ m); carrier, He (50 cm/sec); analysis temperature, 180°C.

Table 1
Raw data of selectivity- analysis relationship for ibuprofen (free acid)*

t°C	(1/T)10 ³	α	ln α
170	2.257	1.06601	0.06392
180	2.208	1.05548	0.05399
190	2.160	1.04501	0.04403
200	2.114	1.03458	0.03399

*Symbols: t , temperature (°C); T , absolute temperature (K); α , selectivity

The (ln α , 1/T) linear correlation is describable by the following equation:

$$\ln \alpha = (0.2091 \pm 0.0025)10^3 1/T - (0.4078 \pm 0.0054) \quad \text{Eq. 1.}$$

$$R = 0.99986, N = 4, SD = 0.00026$$

The excellent linearity of the equation 1 shows that the interaction processes allowing the chiral recognition of ibuprofen belonging to one mechanism, because the equation 1 is linear. The high regression value also underlines that the measurements have high precision values.

As we have demonstrated before [21, 22]:

$$\ln \alpha = \ln (K_B/K_A) = \Delta(\Delta\mu_{BA}^{\circ})/RT = \Delta_{BA}\Delta G^{\circ}/RT \quad \text{Eq. 2}$$

In these relations the symbols are the following: K_B and K_A show the distribution ratios of A and B compounds between the stationary phase and mobile phase; ΔG° , ΔH° and ΔS° represent the normal free enthalpy, enthalpy and entropy changes respectively of the participant chemical processes. The notations $\Delta_{BA}\Delta$ refer to the difference between two forms proposed for separation. In this case the questionable compounds are the enantiomers R , S . The signs of differences are conditioned by the relative values of enantiomer parameters, so hereinafter will appear only with absolute values.

Thermodynamic parameters of chiral selective interactions between ibuprofen (free acids) enantiomers are the following:

$$\Delta_{RS}\Delta H^{\circ} = 0.209 \times 10^3 R = 1.738 \text{ kJ/mol K}$$

$$\Delta_{RS}\Delta S^{\circ} = 0.408 \times R = 3.392 \text{ J/mol}$$

$$\Delta_{RS}\Delta G^{\circ} = \Delta_{RS}\Delta H^{\circ} - T\Delta_{RS}\Delta S^{\circ} = 0.812 \text{ kJ/mol K}$$

The corresponding methyl esters of ibuprofen were graphically illustrated (Fig. 3), chromatograms were measured (Figure 4), calculations (Table 2) were done and equations established (Table 2) with similar to free acids.

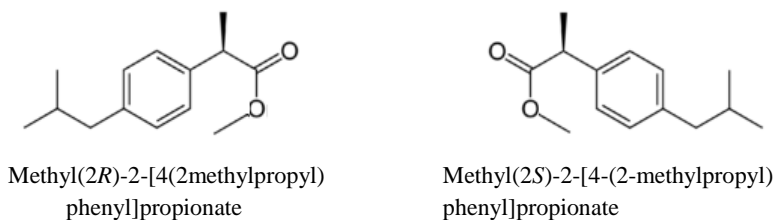


Figure 4

Structure of methyl ester derivatives of ibuprofen enantiomers

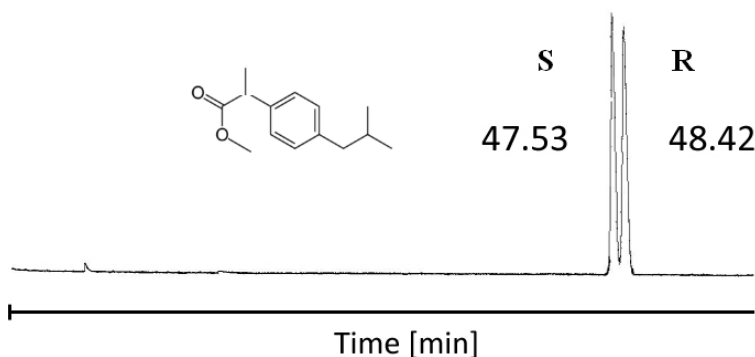


Figure 3

The separation of ibuprofen enantiomers in methyl ester forms with GC.

Parameters: Instrument, Shimadzu QP5000 GC/MS; column, 25 m x 0.22 mm FSOT; stationary phase, Cydex-B (0.25 μm); carrier, He (50 cm/sec); analysis temperature, 110°C.

The peaks of ibuprofen-methyl ester had excellent symmetrical peak shapes. The quantitative determination of secondly eluting isomer can be done without difficulties even if it had only 0.1 % ratio at 100°C. The symmetrical peaks of methyl esters showed high efficiency (4570 theoretical plates/meter), but the tailings of free acids produced broader, less efficient peaks (2150 theoretical plates/meter).

Table 2

Raw data of selectivity- analysis relationship for ibuprofen methyl ester

$t^{\circ}\text{C}$	$(1/T)10^3$	α	$\ln \alpha$
100	2.681	1.02829	0.02790
110	2.611	1.02170	0.02147
120	2.545	1.01700	0.01686
130	2.481	1.01260	0.01252

$$\ln \alpha = (0.0763 \pm 0.0042)10^3/T - (0.177 \pm 0.011) \quad \text{Eq. 3}$$

$$R = 0.9971 \quad N = 4 \quad SD = 0.00060$$

Thermodynamic parameters of chiral selective interactions between ibuprofen methyl esters enantiomers are the following:

$$\Delta_{RS}\Delta H^{\circ} = 0.0763 \times 10^3 R = 0.634 \text{ kJ/mol K}$$

$$\Delta_{RS} \Delta S^{\circ} = 0.0772 R = 0.642 \text{ J/mol}$$

$$\Delta_{RS}\Delta G^{\circ} = \Delta_{BA}\Delta H^{\circ} - T \Delta_{BA} \Delta S^{\circ} = 0.459 \text{ kJ/m}$$

Comparing the results, the free acid shows much higher selectivity ($\Delta_{RS}\Delta G^{\circ} = 0.812 \text{ kJ/mol K}$) than in the case of methyl ester ($\Delta_{RS}\Delta G^{\circ} = 0.459 \text{ kJ/mol K}$). Similar relative values are also observed at the thermic effects and entropic phenomena. But, at comparison, it must observe that the working temperatures differ to a great extent (nearly to 100°C). The differing polarity of free acid and ester is decisive. Most likely, the H-bridge interactions of free acids with the cyclodextrins are supplementary key interactions from the point of view of chiral recognitions.

Esters with longer alky chains or branching (e.g. ethyl, propyl, isopropyl) probably result in less selectivity than methyl ester, similar to amino acids, and pyrethroid acids [24].

Other derivatives of cyclodextrins have been also applied for chiral GC separation of ibuprofen [25, 26, 27]. Comparing the results of different separations, the permethylated- β -cyclodextrin based stationary phases show the highest selectivity toward the free acid of ibuprofen.

It is very edifying to compare the results to those from the literature by CE obtained for the same enantiomer pairs [28]. The effect of temperature on the electrophoretic chiral separation of ibuprofen with β -CD was investigated. Ibuprofen has a temperature independent pK_a value of 4.36. At that same temperature ($t = 25^{\circ}\text{C}$), formation constants K_1 for the uncharged enantiomers (at pH 4.20) are approximately just the same ($10^4 M$). The formation constant K_2 for the charged form, determined at pH 6.55, where only interaction between the fully charged ibuprofen and β -CD takes place, is $5.10^3 M$ for both isomers. From the temperature dependence of these chiral formation constants, we have calculated the corresponding thermodynamic parameters, and the results obtained we have compared to our results (Table 3).

Table 3

The effect of temperature on the chiral interaction parameters between ibuprofen and β -CD at ionic strength 10.0 mMol / L [28]

t ($^{\circ}\text{C}$)	(1/T)* 10^3 (K^{-1})	K_1^* (M)	ln K_1	K_2^* (M)	ln K_2	d K_1^* (M)	ln d K_1
25	3,354	10124	9.223	5256	8.567	339	5.826
32	3,277	6089	8.714	3550	8.175	213	5.361
40	3,193	3692	8.244	2139	7.668	112	4.718
50	3,095	3011	8.010	1675	7.424	78	4.357

* K_1 denotes the formation constant for the uncharged enantiomer, K_2 represents the formation constant for the charged form, and d K_1 is the difference of the stability constants for the uncharged compound.

In the case of undissociated (uncharged) ibuprofen we have obtained the following regression equation:

$$\ln K_1 = (4,72 \pm 0,71)10^3/T - (6,69 \pm 2,30) \quad \text{Eq. 4}$$

$$R = 0.978 \quad N = 4 \quad SD = 0.137$$

The thermodynamic parameters were calculated from van't Hoff relation:

$$\ln K_1 = -\Delta G^\circ/RT = -\Delta H^\circ/R \cdot 1/T + \Delta S^\circ/R \quad \text{Eq. 5}$$

Wherein the notation is the same as explained earlier.

$$\Delta H^\circ = 4.72 \cdot 10^3 R = 39.22 \text{ kJ/mol K}$$

$$\Delta S^\circ = -6.69 R = -55.59 \text{ J/mol} = -0.056 \text{ kJ/mol K}$$

$$\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ = 39.22 + 273 \times (-0.056) = 54.51 \text{ kJ/mol K}$$

The dissociated (charged) ibuprofen has the following characteristics:

$$\ln K_2 = (4,54 \pm 0,56)10^3/T - (6,69 \pm 1,80) \quad \text{Eq. 6}$$

$$R = 0.985 \quad N = 4 \quad SD = 0.108$$

The thermodynamic parameters:

$$\Delta H^\circ = 4.53 \times 10^3 R = 37695.68 \text{ J/mol K} = 37.70 \text{ kJ/mol K}$$

$$\Delta S^\circ = -6.69 R = -55.60 \text{ J/mol K} = -0.056 \text{ kJ/mol K}$$

$$\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ = 37.70 - 273 \times (-0.056) = 52.98 \text{ kJ/mol K}$$

As it is visible, the values obtained for K_1 are higher, as was the case with K_2 . It can be seen that both K_1 and K_2 decrease monotonously with increasing temperature. The negative sign of ΔH (exothermic effect) indicates a decrease of enthalpy, due to the release of high energy water out of the cyclodextrin cavity, and forming of more stable bonds with complexants. The negative sign of ΔS alludes to a decrease of entropy, due to complex formation, which consequently results in a decrease of the degree of freedom of the components involved in the interaction. As expected, the dominant force for analyte binding arises from enthalpy changes.

There is a similarity between derivatization by ester-formation and the dissociation equilibrium of ibuprofen. The ester and the uncharged forms are most apolar and consequently give rise to most stable inclusion complexes. This conclusion is demonstrated by in the Table 3 recapitulated experimental results.

As expected, the selectivity grows with increasing β -CD concentration and dropping temperature in the measured range. The calculated selectivity maximum in the function of concentration of selector is higher than the saturation concentration of β -CD. In CE, the maximum of the resolution of enantiomers

show a maximum curve in the function of selector concentration according to the Wren equation [29]. Namely the concentration values of selector are in first degree in the numerators, but they are in the second degree in the denominators.

Other derivatives of cyclodextrins have been also applied for chiral separation of ibuprofen in CE [30-33]. Good separations were achieved using amino substituted permethylated 6-monoamino-6-monodeoxy- β -cyclodextrin and heptakis(2,3-dimethyl-6-amino-6-deoxy)- β -cyclodextrin, because the oppositely charged selectors and selectand can show high resolutions in CE [29].

The heptakis(2,3-di-O-methyl-6-amino)- β -CD proved the best selector (Figure 3). Even 0.5 mMol concentration of this selector produced R_s 2.18 value.

The retention or migration orders of the enantiomers are a very important factor in the determination of trace impurities [34, 35]. The tailing of the major peak can mask that of the minor compounds if the minor is coming later. The *S* isomers of ibuprofen elutes first in every instance using cyclodextrin base stationary phases in GC. On the other hand, the migration orders of enantiomers can be reverse in CE according to which isomers the minor components are. The first migrating peak is *R* isomer of ibuprofen using permethylated 6-monoamino-6-monodeoxy- β -cyclodextrin selector [32, 33]. The *S* isomer of ibuprofen is the first migrating peak using heptakis(2,3-dimethyl-6-amino-6-deoxy)- β -cyclodextrin [32]. The selectivity mechanism of the two selectors are the same, but migration directions are opposite with these selector, because the electroosmotic flow is reverse in the case of heptakis(2,3-dimethyl-6-amino-6-deoxy)- β -cyclodextrin.

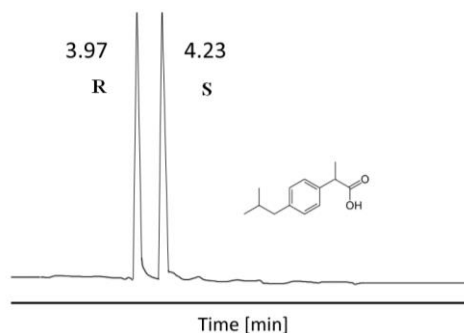


Figure 4

Separation of enantiomers of ibuprofen in free acid form with CE. Parameters: Instrument Hewlett Packard ^{3D}CE; column, 48.5 cm x 50 μ m I.D. FSOT; background electrolyte, Britton Robinson 50 mM (pH5); selector, 0.5 mMol heptakis(2,3-di-O-methyl-6-amino)- β -CD; analysis temperature, 20°C [32].

Conclusion

There is a visible analogy between the derivatization (esterification) and the acid-base dissociation equilibrium. In the both cases the undissociated acid forms give birth with cyclodextrins more selective inclusion complexes, although the fine mechanism of complex formation differs.

Specifically for the chiral separation parameters, it was observed that the stability constant and the selectivity values decrease with increasing temperature, with negative values for both free energy and entropy changes. This means that when optimizing a chiral separation, using the present model, a different operating temperature may lead to different optimized conditions: selectivity will generally be lower at elevated temperatures.

The general rule can be drawn from our results. High resolution can be achieved for enantiomer of nonsteroidal anti-inflammatory drugs using permethylated- β -cyclodextrin containing chiral stationary phase in GC. The free acids show higher selectivity than methyl ester derivatives, but show significant tailing of the peaks. Methyl esters of these enantiomers are recommended derivatives for their GC analysis.

Very high resolutions can be achieved for the nonsteroidal anti-inflammatory drugs using aminosubstituted derivatives of cyclodextrins among CE conditions.

Acknowledgement

This research was partly sponsored by OTKA K72861 grant. The valuable consultations are highly appreciated by Dr. R. Iványi.

References

- [1] R. K. Sharma: Stereochemistry, Discovery Publishing House, New Delhi, 2008
- [2] Sudhir Chandra Pal, Nomenclature of Organic Compounds, Alpha Science International, Pangbourne, 2008
- [3] S. M. Batra, M., A. P. Bhaduri: Chirality and Future Drug Design, Progress in Drug Research 41 (1994) 191
- [4] M. Simonyi, G. Maksay: Stereochemical Aspects of Drug Action II: Optical Isomerism, The practice of Medicinal Chemistry, Academic Press Budapest 1996
- [5] W. Freitag: Contergan, Waxmann Verlag GmbH, Münster 2005
- [6] Department of Health and Human Service of the USA, Food and Drug Administration's Policy Statement for the Development of New Stereoisomeric Drugs, Fed. Regist. 57/ 2 May (1992) 102
- [7] F. Toda (Editor): Enantiomer Separation: Fundamentals and Practical Methods, Kluwer Academic Publisher, Dordrecht 2010
- [8] V. R. Meyer, M. Rais: A Vivid Model of Chiral Recognition, Chirality 1 (1989) 167
- [9] V. Schurig: Enantiomer Separation by Gas Chromatography on Chiral Stationary Phases, J. Chromatogr. A 666 (1994) 111

- [10] Z. Juvancz, V. Kiss, J. Schindler and J. Bálint: Use of Achiral Derivatization to Increase Selectivity and Reverse the Order of Elution of Enantiomers on Chirasil-Dex, *Chromatographia* 60S (2004), S161
- [11] S. Fanali, C. Desiderio, Z. Aturk: Enantiomeric resolution study by capillary electrophoresis: Selection of the appropriate chiral selector, *J. Chromatogr. A* 772 (1997) 185
- [12] P. C. Gøtzsche: Non-steroidal anti-inflammatory drugs, *British Medical J.* 320 (2000) 1058
- [13] M. A. Hamman, G.A. Thompson, S.D. Hall: Regioselective and stereoselective metabolism of ibuprofen by human cytochrome P450, *Biochem. Pharmacol.* 54 (1997) 33
- [14] M. Niemi, P. Neuvonen, Janne Backman: Stereoselective interaction between the CYP2C8 inhibitor gemfibrozil and racemic ibuprofen, *Eur. J. Clin Pharmacol.* 27 (2007) 463
- [15] G. Leising, R. Resel, F. Stelzer, *et al.*: Physical aspects of dexibuprofen and racemic ibuprofen, *J. Clin. Pharmacol.* 36 (1996) 3
- [16] V. Wsól, L. Skálová, B. Szotáková: Chiral inversion of drugs: coincidence or principle?, *Curr. Drug Metab.* 5 (2004) 517
- [17] G. Cretu, M. Ionică, A. F. Dănet, *et al.*: Separation of the enantiomers of ibuprofen by a gas chromatographic–mass spectrometric method, *Acta Chromatographica* 15 (2005) 315
- [18] Jincui Ye, W. Yu, G. Chen, *et al.*: Enantiomeric separation of 2-arypropionic acid nonsteroidal anti-inflammatory drugs by HPLC with hydroxypropyl-beta-cyclodextrin as chiral mobile phase additive, *Biomed. Chromatogr.* 24 (2009) 17
- [19] M. Johannsen: Separation of enantiomers of ibuprofen on chiral stationary phases by packed column supercritical fluid chromatography, *J. Chromatogr. A* 937 (2001) 135
- [20] F. Główka, M. Kraźniewicz: Enantioselective CE method for pharmacokinetic studies on ibuprofen and its chiral metabolites with reference to genetic polymorphism, *Electrophoresis.* 28 (2007) 2726
- [21] B. Tőkés, Z. Juvancz, R. Iványi, S. Vancea, G. Donáth-Nagy, V. Schurig, A. Cârje: Temperature Dependence of Enantiomer Separation Parameters by Gas-Chromatographic and Supercritical Fluid Chromatographic Methods, *Studia Universitatis Babeş-Bolyai Chemia* 54 (2009) 93
- [22] B. Tőkés, Z. Juvancz, R. Iványi, G. Donáth-Nagy, S. Vancea, V. Schurig, A. Cârje: Chemical Structure Dependence of Separation Methods Parameters, *Studia Universitatis Babeş-Bolyai, Chemia* 54 (2009) 103
- [23] K. Blau, G.S. King (Eds.): *Handbook of derivatives for chromatography*, Heyden & Son, N.Y. 1977

- [24] Z. Juvancz, P. Petersson: Enantioselective Gas Chromatography, *J. Microcol. Sep.* 8 (1996) 99
- [25] G. Cretu, M Ionica, A.F. Daner, H. Aboul-Enein, R. Macover, M. Buleandra: Separation of the enantiomers of ibuprofen by gas chromatographic-mass spectrometric method, *Acta Chromatographica* 15 (2005) 315
- [26] http://www.restek.com/chromatogram/view/GC_PH00242 (22.04.2013)
- [27] H. Hühnerfuss, S. Selke, R. Kallenborn, J. Kuhlmann, S. Weigel: Enzymatic transformation of chiral pharmaceuticals in the environment as revealed by enantioselective chromatography, *Natural halogenated and chiral compounds* 68 (2006) 17
- [28] J. C. Reijnga, B. A. Ingelse, F. M. Everaerts: Thermodynamics of chiral selectivity in capillary electrophoresis: separation of ibuprofen enantiomers with β -cyclodextrin, *Journal of Chromatography A*, 792 (1997) 371
- [29] S.A. Wren, R.C. Rowe: Theoretical aspects of chiral separation in capillary electrophoresis: I. Initial evaluation of a model, *J. Chromatogr. A* 603 (1993) 235
- [30] B. K. Patel, M. Hanna-Brown, M. R. Hadley, A. J. Hutt: Enantiomeric resolution of 2-arylpropionic acid nonsteroidal anti-inflammatory drugs by capillary electrophoresis: Methods and applications, *Electrophoresis* 25 (2004) 2625
- [31] R. Hamoudova, M. Pospisilova: Determination of ibuprofen and flurbiprofen in pharmaceuticals by capillary zone electrophoresis, *J. Pharm. and Biomed. Anal.* 41 (2006) 1463-1467
- [32] R. Iványi, L. Jicsinszky, Z. Juvancz: Single isomer permethylated amino cyclodextrins for chiral selective capillary electrophoresis 24th Int. Symp. on Cap. Chromatogr. Las Vegas (2001)
- [33] J. Olsson: New Techniques for Chiral Separations, Dissertation Karlstadt University 2008
- [34] V.Schurig: Separation of enantiomers by gas chromatography, *J. Chromatogr. A* 906 (2001) 275
- [35] L.G. Blomberg, H. Wan: Determination of enantiomeric excess by capillary electrophoresis, *Electrophoresis* 21 (2000) 1940