Int. J. Chem. Sci.: 10(2), 2012, 799-807
ISSN 0972-768X
www.sadgurupublications.com

HPLC SEPARATON OF HYDROPHOBIC ATROPISOMERS

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ABSTRACT

The separation of racemic-N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl)amine, which belongs to the 2-aryl-imino-N-(2-aryl)-thiazoline family, to collect its atropisomers, was studied using reversed-phase high-performance liquid chromatography (RP-HPLC) with hydroxypropyl-gamma-cyclodextrin (HP-γ-CD) as a complexing additive to the racemic mixture (to obtain chiral molecules) and hexane-propan-2-ol as a mobile phase. The effects of mobile phase composition, concentration of HP-γ-CD and time of incubation of the CD-substrate on separation were systematically investigated to establish the optimum resolution conditions.

Key words: 2-aryl-imino-N-(2-aryl)-thiazoline, HP–γ-CD, HPLC, Atropisomeric separation.

INTRODUCTION

Natural cyclodextrins (CD) constitute a family of cyclic oligosaccharides comprising repetitive 6, 7, or 8 glucose units (α-, β-, γ-CD, respectively). The inside of the molecule forms a hydrophobic cavity, enabling it to form molecular inclusion complexes with hydrophobic drugs and components, thus greatly enhancing their solubility in water[1-6]. The polarity of the cavity has been estimated to be similar to that of an aqueous ethanolic

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solution[7]. Due to the chair conformation of the glucopyranose units, the CD molecules take the shape of a truncated cone rather than a perfect cylinder[8]. The aqueous solubility of the natural CDs is much lower than that of the comparable derivative cyclodextrins. In fact, substitution of any of the hydrogen bond-forming hydroxyl groups result in dramatic improvement in their aqueous solubility[9]. CD derivatives such as the hydroxypropyl derivatives (i.e. HP-γ-CD, HP-β-CD and HP-γ-CD), the randomly methylated-CD and sulfobutylether-CD have witnessed extensive applications in pharmaceutical industry[10-14].

Atropisomeric compounds exist naturally in essential molecules such as nucleic acids and alkaloids. The two atropisomers can be exchanged without bond breaking according to a unimolecular process[15,16]. Atropisomers have found a lot of applications as ligand for asymmetric catalysis or as chiral scaffold in asymmetric synthesis[17,18]. Atropisomeric drugs have been also developed for a long time[19]. As well, their synthesis is extensively used in various applications (stains, herbicides, etc)

The 2-aryl-imino-N-(2-aryl)-thiazoline compounds exist as a racemic atropisomers and have been developed and studied due to their biological activities[10,20,21]. The present study reports the separation of the two atropisomers of racemic-N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl)amine, (Fig. 1, a) by reversed phase high-performance liquid chromatography (RP-HPLC) using various native cyclodextrin (α-, β- and γ-CD) and cyclodextrin derivatives, such as HP-α-CD, HP-β-CD and HP-γ-CD. It was found out that HP-γ-CD shows the best separation as shown in Table 1.

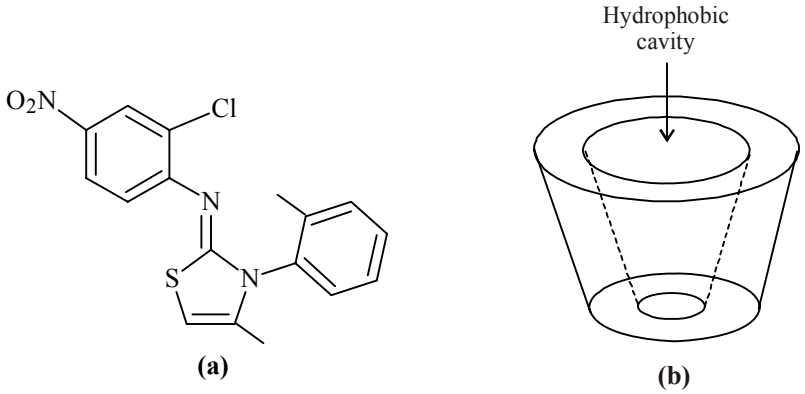

Fig. 1(a): Structure of N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl)amine; (b) truncated cone shape of HP-γ-CD

Table1. Effect of Cyclodextrin on the atropisomeric separation

Experiment	Cyclodextrin	Rs
1	α	0.358
2	HP-α-CD	0.456
3	β	0.941
4	HP-β-CD	0.874
5	γ	1.001
6	HP-γ-CD	1.230

EXPERIMENTAL

Chemicals and reagents

Racemic-N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl)amine was synthesized and purified by Bouchkara et-al[22]. HP-γ-CD, hexane and propan-2-ol of HPLC grade were purchased from Sigma-Aldrich Co. All the solvents used for column chromatography were of HPLC grade and distilled prior to use. Water was purified by triple distillation.

Preparation of solutions

Racemic-N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl)amine was accurately weighted, transferred to volumetric flasks and dissolved in solution of mobile phase 50 : 50 (v/v) hexane-propan-2-ol to make individual stock solutions of 0.1 mmol/L. The stock solution was stored at 4^{o}C and was later diluted with mobile phase to the recommended concentration of 0.01 µmol/L.

Preparation of inclusion complexes

10 µL of 0.25 µmol/L concentration of cyclodextrin and 10 µL of 0.01 µ mol/L of solute were mixed and shaken at the temperature of 25°C to obtain a stable state of solubilization.

Instrumentation

Chromatographic studies were performed on a Schimadzu HPLC system (UFLC) equipped with a thermostated-column device, a degasser and a variable-wavelength UV

detector. The column used for analytical HPLC was C-18 (150 mm × 4.6 mm). The mobile phase was a mixture of hexane and propan-2-ol with a flow rate of 0.5-1 mL/min. The wavelength of UV detector was set at 254 nm and the column was operated at room temperature. The injection volume was 5 µL.

Mobile phase optimization and effect of incubation time

The influence of mobile phase composition was studied, whereby different volumes of hexane ranging from 0% to 100% (v/v) while keeping the HP- γ -CD (0.25 µ mol/L) and substrate (0.01 µ mol/L) concentrations constant.

To determine the effect of time on resolution, the experiment was performed at different incubation times with a flow rate of 1 mL/min while keeping the conditions as described above. The atropisomeric separation ability was evaluated by resolution. The resolutions were based on the average of at least three independent determinations.

Optimization of the HP-γ-CD concentration

The HP-γ-CD concentration effect on the resolution was investigated using 0.01-10 µmol/L of HP-γ-CD in the presence of 0.01 µmol/L of racemic-N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl)amine using a mobile phase composed of 50:50 (v/v) hexane-propan-2-ol.

RESULTS AND DISCUSSION

Separation of atropisomers

The chromatograph of racemic-N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl)amine showed a single peak (Fig. 2) indicating the purity of the substrate. In order to achieve the separation of the atropisomers, 0.25 µmol/L of HP-γ-CD are added to 0.01 µmol/L of the substrate using a mobile phase composed of 50 : 50 (v/v) hexane-propan-2-ol. The atropisomeric separation ability was evaluated by resolution. The result in (Fig. 3) showed the presence of two separated peaks at Rt 1.95 and 2.45 min. corresponding to the two atropisomers.

Effect of mobile phase parameter

Optimization of the mobile phase composition was achieved by testing the different percentages (a range of 0-100%) of Hexane-propan-2-ol. The best results were obtained for a 50% propan-2-ol as described in (Fig. 4). Resolutions less than 0.5 were observed for propan-2-ol percentages below 30% and higher than 70%.

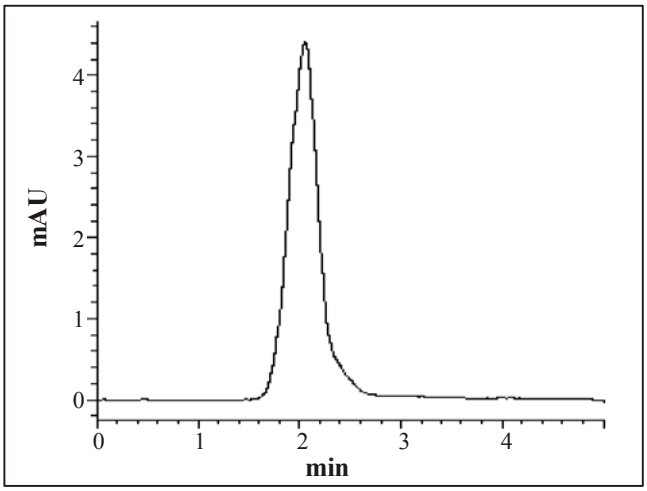

Fig. 2: Chromatogram of racemic-N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl)amine. Flow rate: 1 mL/min. Other chromatographic conditions as in Fig. 3

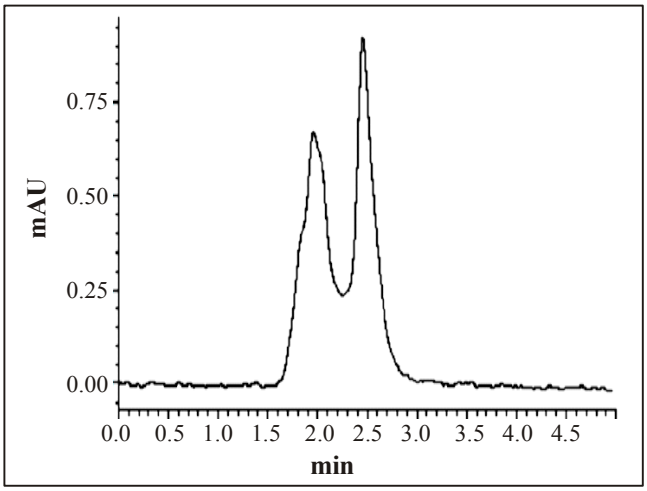

Fig. 3: Separation of atropisomers at 0.25 µM concentration of HP-γ-CD, 0.01 µM concentration of substrate. Chromatographic conditions: Schimadzu HPLC system, (150 mm × 4.6 mm) column. Mobile phase: 50 : 50 (v/v) hexane-prop-2-ol. Flow rate: 0.5 mL/min. Injection volume: 5 µL. Wavelength used for UV detection : 254 nm. At room column temperature

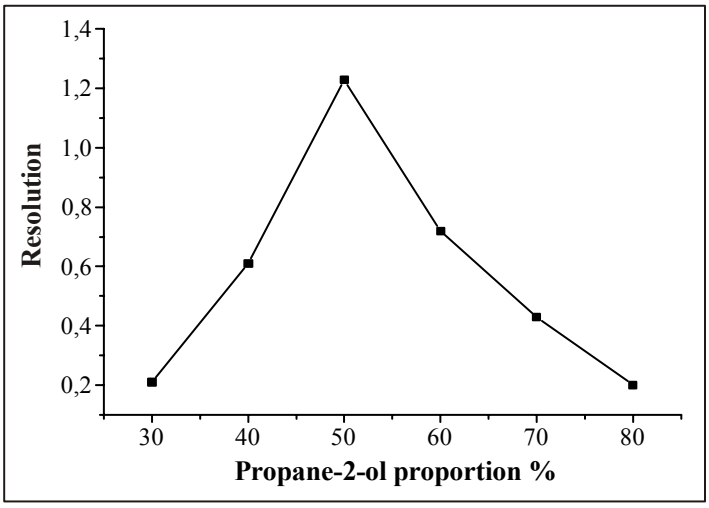

Fig. 4: Effect of propan-2-ol proportion on the atropisomers resolution, 0.25 µmol/L concentration of HP-gamma-CD, 0.01 µmol/L concentration of substrate are used. Flow rate: 1 mL/min. Other operating conditions as in Fig. 3

Effect of time

The effect of time was investigated and, as shown in (Fig. 5), no difference in resolution was observed. This indicated that the formation of the complex was obtained in a very short time. The extreme rapidity of the complex formation may be justified by the inclusion of the phenyl ring only in the hydrophobic cavities of CD molecules.

Effect of HP-γ-CD concentration

Addition of HP-γ-CD (0.01-10 µmol/L) to the substrate (0.01 µmol/L) using a mobile phase composed of 50 : 50 (v/v) hexane-propan-2-ol showed that the best result was obtained using an intermediate concentration of 0.25 µmol/L HP-γ-CD (Table 2). The lower resolutions obtained for higher HP-γ-CD concentrations may be attributed to a decrease in the efficiency of the column.

In this study, several experimental parameters were examined to determine the optimal atropisomeric separation conditions. The cyclodextrin nature (α-, HP-α-CD, β-, HP-β-CD, γ- and HP-γ-CD), the concentration of cyclodextrin, the mobile phase composition and the time of incubation CD-substrate.

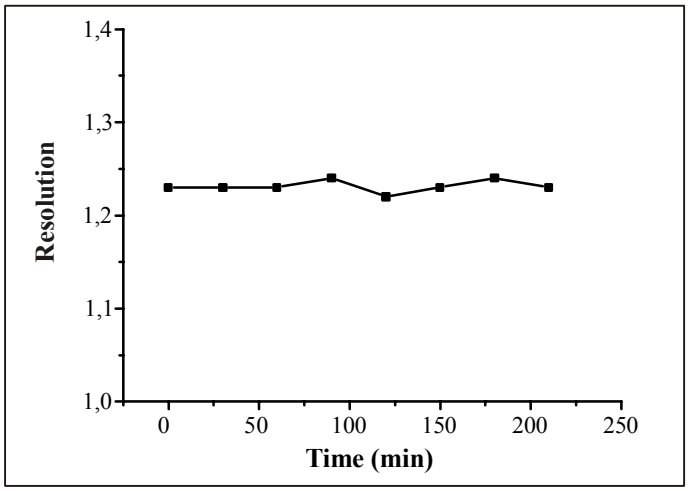

Fig. 5: Effect of time on the atropisomers resolution. 0.25 µmol/L concentration of HP-gamma-CD, 0.01 µmol/L concentration of substrate are used. Flow rate: 1 mL/min. Other operating conditions as in Fig. 3

Table 2: Effect of HP-γ-CD concentration on the atropisomeric resolution

Substrate (µmol/L)	HP-γ-CD (µmol/L)	Resolution
0.01	0.01	0.54
0.01	0.03	0.68
0.01	0.07	0.78
0.01	0.25	1.23
0.01	0.5	1.056
0.01	1	0.92
0.01	10	0.82

The best stereoselective interactions for the substrate were obtained with HP-γ-CD which may be related to the capacity and the polarity of HP-γ-CD cavity. α-, HP-α-CD, β-, and HP-β-CD seem to have a small cavity that prevented the substrate inclusion phenomenon from taking place. The large size of the cavity in the case of γ-CD encourages the exit of the substrate after inclusion. Only HP-γ-CD shows a persistent inclusion of the substrate, due to the presence of the hydroxypropyle groups. These results confirm that the predominating separation mechanism of CD for 2-aryl-imino-N-(2-aryl)-thiazoline

compounds was based on the phenomenon of CD-substrate inclusion, where a transient diastereomeric complex is formed between the CD and the substrate[23-24].

CONCLUSION

This is the first report of atropisomeric separation of racemic-N-[(2Z)-4-methyl-3-(2-methylphenyl)-1,3-thiazol-2(3H)-ylidene]-N-(2-chloro-4-nitrophenyl)amine with HPLC using HP-γ-CD as chiral additive.

The method used is based on the addition of the cyclodextrin with the substrate. A complex cyclodextrin-substrate is formed, and passed on a stationary phase of a RP-HPLC. The high solubility of HP-γ-CD and the substrate in the mobile phase of 50 : 50 (v/v) hexane-propan-2-ol facilitates the optimization of the chromatographic conditions. The separation was easily achieved and pure atropisomers were obtained.

The chromatographic conditions described herein provide a novel, rapid and reliable approach for the separation and the analysis of atropisomers from synthesized samples. Supplementary studies using NMR is recommended to confirm the inclusion of the analyte inside the hydrophobic cyclodextrin cavity.

ACKNOWLEDGEMENT

This research is supported by the management committee of scientific research at the Lebanese University.

REFERENCES

1. I. W. Wainer and D. Drayer, Drug Stereochemistry, Analytical Methods and Pharmacology, Marcel Dekker, New York (1998).
2. J. Hermansson and C. Von Bahr, J. Chromatogr., B, **221**, 109-117 (1980).
3. J. Hermansson, Acta Pharm. Suec., **19**, 11-24 (1982).
4. J. A. Thompson, J. L. Holtzman, M. Tsuru and C. L. Lerman, J. Chromatogr., A, **238**, 470- 475 (1982).
5. N. Matsuo and N. Ohno, Tetrahedron Lett., **26**, 5533-5534 (1985).
6. Y. Terao, M. Murata, K. Achiwa and T. Nishino, M. Akamatsu and M. Kamimura, Tetrahedron Lett., **29**, 5173-5176 (1988).
7. J. Szejtli, Pure Appl. Chem., **76(10)**, 1825-1845 (2004).

8. M. E. Brewster and T. Loftsson, Adv. Drug Delivery Rev., **59(7)**, 645-666 (2007).

9. K. Uekama, Adv. Drug Delivery Rev., **36(1)**, 101-123 (1999).

10. T. Loftsson and M. E. Brewster, J. Pharm. Sci., **85(10)**, 1017-1025 (1996).

11. K. Uekama, F. Hirayama and T. Irie, Chem. Rev., **98(5)**, 2045-2076 (1998).

12. J. Szejtli, Cyclodextrins and Their Inclusion Complexes, Akademiai Kiadó, Budapest, (1982) p. 296.

13. R. A. Rajewski and V. J. Stella, J. Pharm. Sci., **85(11)**, 1142-1168 (1996).

14. T. Irie and K. Uekama, J. Pharm. Sci., **86(2)**, 147-162 (1997).

15. E. Tobler, M. Lämmerhofer, G. Mancini and W. Lindner, Chirality, **13(10)**, 641-647 (2001).

16. T. Sugimura, N. Matsushita, K. Minokami and S. Kurita, Tetrahedron, **63(8)**, 1762-1769 (2007).

17. H. Kumobayashi, T. Miura, N. Sayo, T. Saito and X. Zhang, Synlett (Special Issue), 1055-1064 (2001).

18. T. Adler, J. Bonjoch, J. Clayden, M. Font-Bardia, M. Pickworth, X. Solans, D. Sole and L. Vallverdu, Org. Biomol. Chem., **3(17)**, 3173-3183 (2005).

19. A. Mannschreck, H. Koller, G. Stühler and M. A. Davies, J. Traber, Eur. J. Med. Chem. Chim. Therap., **19**, 381 (1984).

20. C. Roussel, N. Vanthuyne, N. Shineva and M. Bouchekara, A. Djafrie and Arkivoc, 28-41 (2008).

21. C. Roussel, N. Vanthuyne, M. Bouchekara, A. Djafri, J. Elguero and I. Alkorta, J. Org. Chem., **73(2)**, 403-411 (2008).

22. M. Bouchekara, A. Djafrie, N. Vanthuyne and C. Roussel, Arkivoc, 72-79 (2002).

23. H. Lamparczyk and P. K. Zarzycki, J. Chromatogr., A, **668(2)**, 413-417 (1994).

24. G. Tárkányi, J. Chromatogr., A, **961(2)**, 257-276 (2002).

Accepted : 05.12.2011