Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1041 (2004) 187–193
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Chromatographic behaviour of naproxen–cyclodextrin complexes Stationary phase C_8 alkyl chain as competitor for the drug release from cyclodextrin cavity

S. Rozou, E. Antoniadou-Vyza*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece

Received 5 March 2004; received in revised form 3 May 2004; accepted 5 May 2004

Abstract

Cyclodextrins are known to alter the absorptivity of the guest molecules, therefore, analytical methods that are based on the spectrophotometric data present accuracy problems. In this work, using RP-HPLC methods for naproxen–cyclodextrins quantitation, extensive analytical inaccuracies are detected. Competitive complexation technique is utilised in an attempt to develop an analytical method enabling the determination of naproxen as a free drug. For this reason, stationary phases with silica ligands that can function as competing agents were used, thus contributing to the drug release. The release of the drug from cyclodextrins complexes is achieved by modification of the thermodynamic parameters that determine the stability constant, by changing: the interactions with the mobile phase components (e.g. pH, organic modifier, competitive agents) and the interactions with the stationary phase ligands (C_8). After studying the parameters affecting the interaction between the alkyl-chain C_8 and naproxen:cyclodextrin complexes, we developed and validated a new specific method for the accurate determination of the drug. Consecutive accumulation of the cyclodextrins molecules on the stationary phase was studied.

Keywords: Complexation; Silica, C_8 bonded; Nonsteroidal anti-inflammatory drugs; Naproxen; Cyclodextrins

1. Introduction

Naproxen [*d*-2-(6-methoxy-2-naphthyl) propionic acid] is a non-steroidal anti-inflammatory drug, which is known to form 1:1 inclusion complexes with β-cyclodextrin and its synthetic derivatives [1–3]. Various analytical methods are used to determine naproxen in the presence of cyclodextrins, including UV detection [1,4,5], fluorimetry [6], NMR [3] and ion-selective electrodes [7].

Cyclodextrins are a well-known group of water-soluble macromolecules, which form inclusion complexes with a great variety of guest molecules. This property is attributed to their shape, which is like a truncated cone, and to their relatively hydrophobic cavity. Cyclodextrins are formed from 6, 7 or 8 D-glucopyranose molecules, linked with α-1,4-glucositic bonds. The sceletal hydrogens and the glucosidic oxygen bridges are located inside the cavity. The non-bonding electron pairs of these oxygens are oriented towards the internal part of the cavity, thus producing a high electron density environment and lending to it some Lewis base characteristics.

The formation of the inclusion complexes depends primarily, on the molecular complementarity, and the substituents of the penetrating part of the guest and the cyclodextrins' molecules. The effect of several other factors such as the temperature, the solvent's polarity and ion strength, the presence of charged species, the molecular conformations and the guest orientation, can also be considered as critical for the strength of the complex stability.

The stability of drug:cyclodextrin complexes, as a binary system equilibrium, is extensively investigated and the studies focus on the binding constant (K_C) calculation and the related thermodynamic parameters (ΔC_p°, ΔH°, ΔS°, ΔG°. Nevertheless, it is difficult to find information for ternary,

* Corresponding author. Tel.: +30-210-7274520/7274822; fax: +30-210-7274747/3625332.
E-mail address: vyza@pharm.uoa.gr (E. Antoniadou-Vyza).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.05.002

quaternary or multicomponent systems with several coexisting equilibria, such as chromatographic systems including free and complexed molecules.

Cyclodextrins are known to alter the absorptivity of the guest molecules [8,9], therefore, analytical methods that are based on the spectrophotometric data present accuracy problems. According to the existing literature, naproxen shows different UV absorption and fluorimetric profile in the presence of cyclodextrins, in relation to the free drug [6,10]. These discrepancies of the analytical methods were not taken into consideration by various researchers. As a result of the utilised non-specific analytical methods, a great number of different K_C values corresponding to a specific inclusion complex can be found in the literature [4,10,11].

All these problems generate the need of an analytical method that could be able to determine naproxen as free drug, quantitatively and accurately. Reversed-phase HPLC is the most common technique used in an analytical laboratory, hence it was considered more appropriate to focus on the development of a relevant method. From the analytical point of view, the drug fraction released from the drug:cyclodextrins complexes can be considered as a critical parameter. A detailed study of the literature showed that no attention was given before to similar cases.

In chromatographic systems, the already investigated drug–cyclodextrins binary interactions can be affected from the presence of other components, which consist part of multiple equilibria, and should be studied in connection to them. In such a binary system, the addition of any interfering component can modify the already existing equilibria. As a result, significant differences in the adsorption, partitioning and exclusion phenomena show up, hence the overall chromatographic behaviour changes.

In the course of our previous investigations [8], we realised that, in order to achieve the release of the drug from the cyclodextrins' cavity during the chromatographic analysis, complexation thermodynamics must be altered in such a way, that the binding of any compound competitor to cyclodextrins should be favoured and the drug's inclusion in the cyclodextrins' cavity unfavoured. Any chemical reagent, which shows better binding affinity towards the cyclodextrin's cavity can be used as displacer of the drug from cyclodextrin [12].

Adsorption phenomena of cyclodextrins on reversed-phase stationary phases (RP18, RP8) can be found in previous references [13,14], but for the insertion phenomena of the RP18 alkyl chains in the cyclodextrins' cavity only a theoretical approach with the aid of molecular modelling calculations is reported [15]. This differs significantly from the crystallographic findings [16,17].

In the present work, a novel approach was attempted. Stationary phases with silica ligands, which can function as competing agents, were used in order to facilitate the drug release from the cyclodextrins' cavity. The parameters affecting the interaction between the alkyl-chain C_8 and naproxen:cyclodextrin complexes were studied and a new specific RP-HPLC method for the accurate determination of the drug was developed and validated. Consecutive accumulation of the cyclodextrins on the stationary phase was investigated.

2. Experimental

2.1. Chemicals

HPLC-grade methanol and water were purchased from Carlo Erba (Modena, Italy). The mobile phases were vacuum filtered and degassed through 0.45 μm pore PTFE membranes with the aid of the Millipore filtration system (Millipore, Milford, MA, USA). The buffers used during the development of the method were prepared with phosphate salts. Naproxen, β-cyclodextrin (βCD), randomly methylated β-cyclodextrin (MeβCD) and 2-hydroxypropyl-β-cyclodextrin (2-HPβCD) (molar degree of substitution 0.8) were purchased from Sigma (St. Louis, MO, USA). βCD polymer CY2009 was purchased from Cyclolab (Cyclodextrins R&D, Budapest, Hungary).

2.2. Equipment

Experiments were carried out using a Waters high-performance liquid chromatograph (Alliance 2690 Separation Module, Waters, Milford, MA, USA), equipped with a photodiode array detector, a constant temperature oven and an autosampler. The chromatographic columns were: a LiChrospher C_8 150 mm × 4.6 mm (10 μm particle size) and a LiChrospher C_{18} 150 mm × 4.6 mm (10 μm particle size), both purchased from MZ (Mainz, Germany). Millenium 32 was used as software facility (Waters).

3. Methods

3.1. Preparation of the complexes

Unbuffered aqueous solutions of 1.7×10^{-5} M naproxen and 9.2×10^{-4} M βCD, 2-HPβCD and MeβCD were used as initial samples. Aliquots of naproxen solution and each individual cyclodextrin solution were combined to prepare the final solutions with molar ratios 1:1, 1:2, and 1:40. The solutions were shaken in a constant temperature water bath, at 25 ± 0.1 °C, until equilibrium was reached (6 days). The equilibrium was evidenced by continuous monitoring of the UV absorption, a feature strongly affected from the extent of complexation.

3.2. High-performance liquid chromatography

The analytical method was developed on the basis of naproxen physicochemical properties and on previous

knowledge [8,9]. The mobile phase of the resulting method consisted of methanol–buffer pH 7.0 (50 mM, KH_2PO_4) (70:30, v/v). The flow-rate was set to 1.0 ml/min and the wavelength at 230 nm. This method was further validated in order to be applied on a routinely basis. The validation experiments were repeated with samples of all used cyclodextrins and stoichiometric ratios. The correlation coefficient of the linearity tests was at least 0.999 in all cases. The R.S.D. of naproxen assay in six replicate solutions of the complexes (precision) was always <2.0%.The percent relative errors during the recovery experiments (accuracy) did not exceed 2.0%.

4. Results and discussion

In the present study, initially the UV-Vis spectra of naproxen in the presence of various cyclodextrins are recorded. In Fig. 1, spectra of naproxen–HPβCD solutions are only shown, for brevity reasons. As it is obvious from Fig. 1, cyclodextrins strongly affect the absorptivity of the drug, and any attempt to determine naproxen quantitatively by UV detection ended in relative errors up to 30%. According to our previous knowledge [8], a specific procedure must be followed, in an attempt to determine naproxen in its free form and obtain, thus, accurate results.

The chromatographic system in the case of supramolecules is very particular, and different components contribute to several equilibria. In Fig. 2, a multicomponent system, including free and complexed molecules, is depicted.

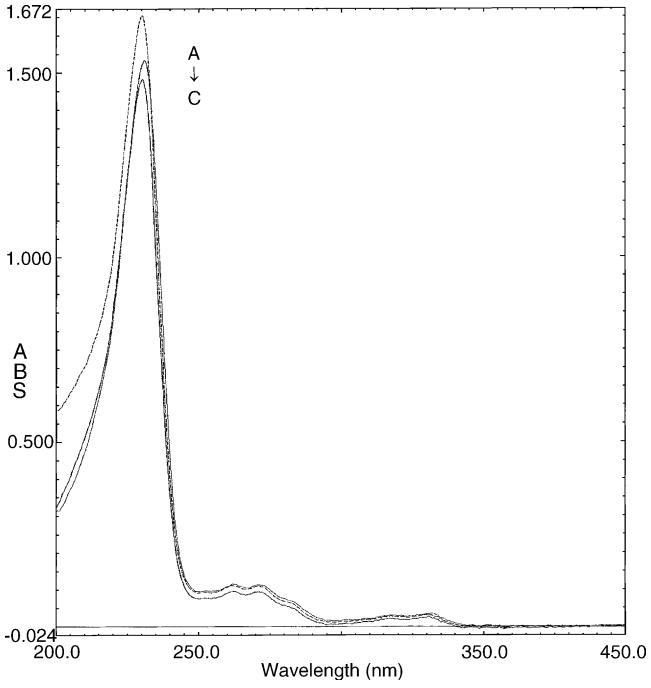

Fig. 1. UV absorption spectra of 1.73×10^{-5} M naproxen solutions with various HPβCD concentrations. Read from A to C: 1.73×10^{-5} M, 6.92 $\times 10^{-4}$ M and 0.

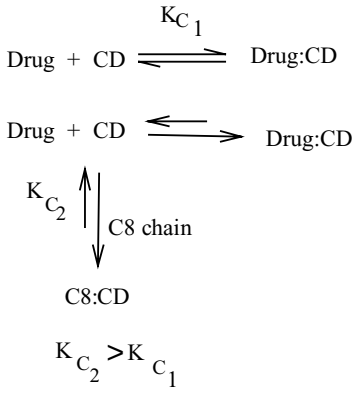

Fig. 2. Schematic representation of a multicomponent system consisting of the free drug, the cyclodextrin (CD) and their inclusion complexes (drug–CD). Multiple equilibria take place between the chromatographic system components, e.g. between cyclodextrins and the ligands (C_8) of the stationary phase.

The use of molecules or part of molecules with better binding affinity towards the cyclodextrins' cavity will destabilise the existing several equilibria, shifting them towards the direction of the free drug.

Any alteration of the mobile phase pH, selected in order to obtain the drug in the form (neutral or ionic) that binds more weakly with the cyclodextrins, will affect the value of the binding constant K_C.

Finally, by modifying the column temperature, the stability constant changes accordingly. The higher the temperature, the lower the K_C value according to the van't Hoff equation.

4.1. Effect of mobile phase pH on a C_{18} chromatographic column

The stability constant of the non-ionised form is always greater than the respective constant of the ionised form.

$$CD + HNX \overset{K_{C\,CD:HNX}}{\rightleftharpoons} CD : HNX$$

$$CD + NX^- \overset{K_{C\,CD:NX^-}}{\rightleftharpoons} CD : NX^-$$

$$K_{C\,CD:HNX} > K_{C\,CD:NX^-}$$

Therefore, the objective of an analytical method should be to ionise the drug, in order to obtain more free drug molecules, a consequence of the smaller K_C ($K_{C\,CD:HNX} > K_{C\,CD:NX^-}$).

In the case of naproxen, the effect of the mobile phase pH was studied first on a C_{18} chromatographic column. The tested pH varied from 3.0 to 8.0 and it was observed that the calculated relative errors between theoretical and experimental values were significantly high in the presence of all the cyclodextrins used in the experiment. The relative errors varied from 0.12 to 26.78% {% relative error is defined as $E_r\% = (E_r/\mu) \times 100 = [(x_i - \mu)/\mu] \times 100$, where x_i is the

calculated concentration of naproxen and μ the theoretical concentration}.

At higher than pH 5.0 values, where naproxen is ionised, the equilibrium started to shift towards the free drug. Especially in the case of naproxen–HPβCD complexes this observation is more pronounced and this might be due to the ionisation of naproxen and the rearrangement of the existing multiple hydrogen bonds in this specific system, which are affected from the pH variations. It can be suggested that the less extensive hydrogen bonding in the naproxen complexes with βCD and MeβCD is the reason for not obtaining any better results by pH modifications.

The more alkaline pH values were considered as the most appropriate for weakening the stability constant and minimising the extent of errors. This fact certifies once again that the K_C of the HNX–CD is greater than the one of NX^-–CD.

4.2. The interaction with C_8 alkyl chain

In the course of the analysis of naproxen:cyclodextrin complexes, it was remarked that the drug was detected accurately only when, in combination with certain chromatographic conditions, a stationary phase with C_8 alkyl chain was used. This can be ascribed to the greater affinity of the cyclodextrins' cavity towards C_8 chain in comparison to naproxen. After the unsuccessful similar experiments with C_{18} alkyl chains, it can be suggested that the thermodynamics of the system did not favour the inclusion of this chain in the cyclodextrins cavity. Bielejewska et al. [13] mentioned that only heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin is adsorbed on C_{18} stationary phases. It is probable that an alkyl chain with 8 carbon atoms can be more easily accommodated in β-cyclodextrin cavity, than an alkyl chain with 18 carbons [15,16]. Several reasons can be suggested. (a) The smaller and more flexible chains can obtain different conformations inside the cavity, thus increasing the entropy of the system and consequently the stability of the new C_8–cyclodextrins complex. The retention of cyclodextrins on the stationary phase causes the shift of the equilibrium towards the free drug. (b) The long C_{18} chains are usually tangled up, thus creating steric hindrance for complexation.

To evaluate the extent of the above-mentioned competition observed with the C_8 alkyl chain, solutions of the same complexes diluted in mobile phase were measured at the UV-Vis spectrophotometer. The calculated relative errors were significantly high. The measurements were taken at regular time intervals and it was noteworthy that the results for a period of at least 4 h did not alter. Thus, the interaction between complexes and the stationary phase components was suggested to be the crucial parameter for the attempted separation.

This was further proved by accumulation phenomena observed during the following experiment. After 12 h of successive injections of the complexes' solutions, the retention time of naproxen was shifted to lower values and its peak was distorted (Fig. 3). This can be explained by the fact that the active sites of the stationary phase were occupied by the accumulated cyclodextrins, thus allowing a quantity of the drug to flow through the column without interacting with them.

Finally, the stationary phase was washed with 10–20 column volumes of methanol and cyclodextrins were flushed from the active sites.

4.3. Effect of mobile phase pH on a C_8 chromatographic column

By keeping the entire chromatographic conditions stable and changing only the pH of the mobile phase, it is obvious that the ionisation of naproxen changes. Apart from pH 7.0, which was adopted for the final analytical method, the mobile phase pH was also adjusted to 3.0 and 5.0. In all cases, naproxen was determined quantitatively, which means that probably the thermodynamics of the system is not significantly affected by the pH changes. The interaction between cyclodextrins and the C_8 alkyl-chain seems to be dominant over the interaction between cyclodextrins and naproxen.

4.4. Organic modifier concentration effect

The concentration of methanol in the mobile phase was considered a significant parameter for the success of the analysis. Ratios from 50 to 80% were used in an attempt to investigate the contribution of methanol to the complex dissociation. It was concluded that concentrations higher than 60% lead to a shift of the equilibrium towards free naproxen (Table 1). This finding might be attributed to the following two reasons: (a) the presence of hydrophobic interactions between naproxen and the cyclodextrins cavity, which are influenced by the medium characteristics, such as polarity and hydrophobicity; and (b) the competitive complexation of methanol with the cyclodextrins, although methanol shows weaker binding affinity than naproxen. The large excess of methanol obviously contributes to this phenomenon.

At methanol concentrations bigger than 70% the relative errors showed some fluctuations (Table 1), which can be ascribed to a probable self-dimerisation or aggregation of the cyclodextrins or their complexes in a medium of enhanced hydrophobicity, inhibiting, thus, the extraction of the free drug.

The retention of naproxen is independent of cyclodextrin concentration as it can be seen by the calculated k' values (Fig. 4). It was observed that, in all cases, the capacity factor of the free drug (k'_0) had the same values as k' of the drug in the presence of cyclodextrins. Since the capacity factor is dependent on the distribution of the drug between the mobile and the stationary phase (Eq. (1)), it can be concluded that this distribution remained constant in the presence and in the absence of the cyclodextrins (as long as the temperature and the concentration of methanol are kept stable).

$$k' = \frac{C_s V_s}{C_m V_m} \quad (1)$$

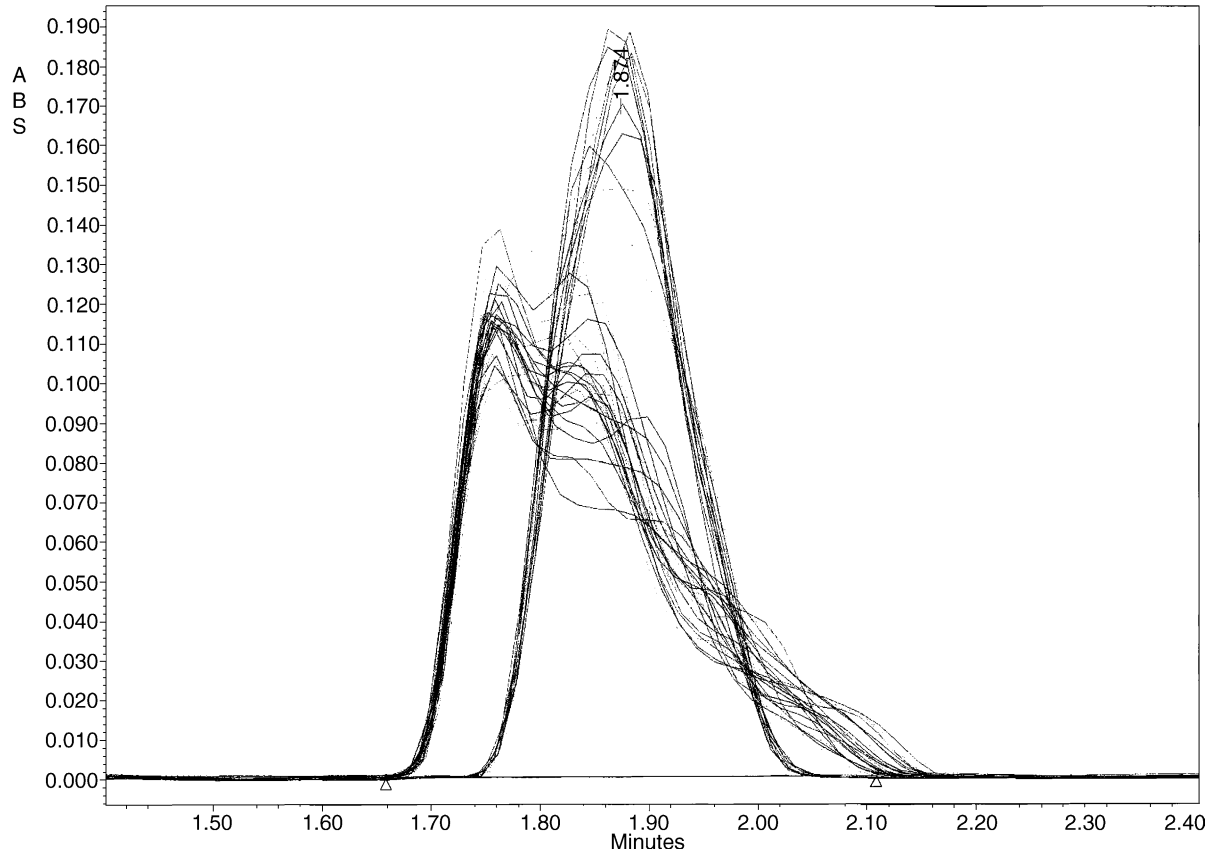

Fig. 3. Chromatogram showing the characteristic distortion of naproxen peak after consecutive injections of its complex with cyclodextrins in the C_8 column.

It is also observed that $\ln k'$ decreased exponentially (Eq. (2)) along with the increase of the methanol concentration, in agreement to the equation:

$$\ln k' = D + A\varphi^2 + B\varphi + C\sqrt{\varphi} \quad (2)$$

where k' denotes capacity factor, φ the volume fraction of the organic modifier in the mobile phase, A, B, C and D the coefficients resulting from multiple regression analysis.

4.5. Temperature effect

Temperature was altered within the specified limits of the instrumentation used. Three different column temperatures,

Table 1
The calculated percent relative errors of the HPLC method applied for the analysis of naproxen in the presence of cyclodextrins, at various methanol concentration in the mobile phase

	Relative errors						
	50% MeOH	55% MeOH	60% MeOH	65% MeOH	70% MeOH	75% MeOH	80% MeOH
NX–βCD (1:1)	−4.60	−3.79	−7.26	−2.41	−0.66	−1.31	+1.34
NX–βCD (1:2)	−3.45	+0.50	−4.73	−0.16	+1.28	−0.12	−0.22
NX–βCD (1:40)	+2.44	+2.18	−8.14	−1.08	+0.81	−1.39	+1.79
NX–HPβCD (1:1)	−3.32	+0.92	−7.42	−2.67	−1.55	+1.28	+0.22
NX–HPβCD (1:2)	−6.47	+0.22	−11.05	−2.71	+0.61	−1.99	+0.03
NX–HPβCD (1:40)	−6.85	+3.61	−3.01	−0.32	+0.43	+0.51	+0.94
NX–MeβCD (1:1)	−4.58	+1.36	−11.79	−1.47	+0.18	−0.83	+1.45
NX–MeβCD (1:2)	−5.29	−0.82	−13.42	−3.54	−1.31	+1.13	+0.74
NX–MeβCD (1:40)	+2.87	+3.55	−1.73	+3.57	−1.40	−1.78	+0.22
NX–βCDpol (1:1)	−6.78	+1.14	−10.86	−2.40	−0.12	+1.51	+1.33
NX–βCDpol (1:2)	−4.59	+1.63	−9.59	−2.01	+0.04	−1.75	−0.52
NX–βCDpol (1:40)	+0.83	+3.68	+0.16	+2.70	+0.64	+1.47	−0.17

Chromatographic conditions: LiChrospher C_8 150 mm × 4.6 mm, 10 μm at 35 °C, methanol–buffer pH 7.0 in varying ratios, flow-rate 1.0 ml/min, wavelength 230 nm.

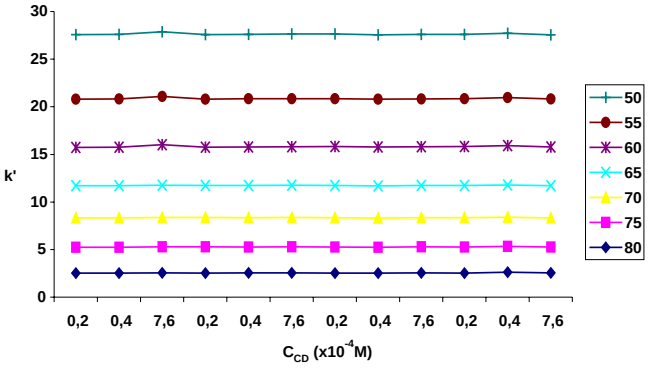

Fig. 4. Profile of naproxen capacity factor values vs. the concentration of cyclodextrins in its solutions.

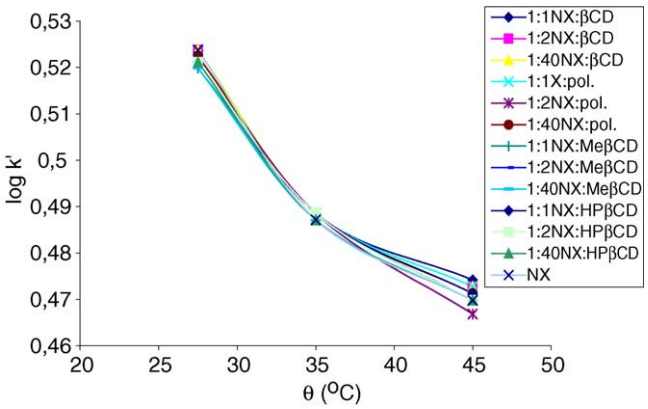

Fig. 5. The effect of column temperature on the capacity factor values of naproxen.

27.5, 35 and 45 °C, were selected. The chromatographic parameters, presented in Table 2, were evaluated.

It was observed that by increasing the column temperature, the capacity factor, k', decreased, which is expected to happen because of the viscosity decrease. This fact can also be ascribed to the 'loosening' of the hydrogen bonds between naproxen anion and the free silanols of the stationary phase.

From Fig. 5 it can also be observed that $\log k'$ shows a deviation from the expected linearity up to 45 °C. A possible interpretation might be the significant change in stationary phase's alkyl chain activity. As it is reported for C_8 alkyl chains, a transition temperature of 45 °C, exists, above which, conformational modifications were expected [18]. Therefore, the interacting with cyclodextrins active sites become fewer and k' is expected to increase when the temperature increases above 45 °C.

At the temperature of 35 °C it is observed that the capacity factor of free naproxen obtains the same value as the one calculated in the presence of cyclodextrins. At the other temperatures of the experiments these values differ, allowing us to suggest that this is the consequence of the not complete extraction of the drug from cyclodextrins' cavity.

Moreover, as temperature increased, naproxen peak became broader, and it can be suggested that the interactions of naproxen with the stationary phase were stronger, as a consequence of the increasing energy of the molecules.

From the evaluation of the calculated relative errors, the temperature of 35 °C proved to be the most appropriate for this specific analysis. Possibly the temperature alterations affected the complex structure, mainly in the systems where hydrogen bonds contribute to complex formation.

Table 2
Capacity factor and theoretical plates of naproxen and naproxen complexes, in different column temperatures

	k' values			Theoretical plates		
	27.5 °C	35 °C	45 °C	27.5 °C	35 °C	45 °C
NX–βCD (1:1)	3.34	3.08	2.98	4090	4322	4592
NX–βCD (1:2)	3.34	3.08	2.97	4057	4349	4732
NX–βCD (1:40)	3.34	3.08	2.97	4107	4318	4359
NX–βCDpol (1:1)	3.33	3.08	2.97	4078	4223	4418
NX–βCDpol (1:2)	3.33	3.08	2.93	4205	4367	4317
NX–βCDpol (1:40)	3.33	3.08	2.96	4138	4267	4494
NX–HPβCD (1:1)	3.32	3.08	2.96	4199	4385	4552
NX–HPβCD (1:2)	3.32	3.08	2.95	4105	4365	4349
NX–HPβCD (1:40)	3.32	3.07	2.95	4160	4398	4413
NX–MeβCD (1:1)	3.31	3.08	2.95	4211	4393	4572
NX–MeβCD (1:2)	3.31	3.07	2.95	4149	4209	4540
NX–MeβCD (1:40)	3.31	3.07	2.95	4104	4333	4435
NX	3.34	3.07	2.95	4144	4341	4284

Chromatographic conditions at various temperatures: LiChrospher C_8 150 mm × 4.6 mm, 10 μm, methanol–buffer pH 7.0 (70:30, v/v), flow-rate 1.0 ml/min, wavelength 230 nm.

5. Conclusions

The chromatographic behaviour of drug:cyclodextrins complexes in the HPLC system is affected from the multiple equilibria within this multicomponent system. In this case, the quantitation of the free drug from its formulation with cyclodextrins can be particularly complicated and a specific analytical method should be developed.

The liberation of the drug is achieved by modifying the thermodynamic parameters that determine the binding, by changing: the existing interactions with the mobile phase components (e.g. pH, organic modifier, competitive agents) and the interactions with the stationary phase ligands (C_8).

Naproxen was quantitatively extracted from its cyclodextrins complexes only when molecules or parts of them showing better affinity towards the cavity were present in the chromatographic system. This fact indicates the complexity of the cyclodextrins intermolecular equilibria. The pH, the medium lipophilicity or the temperature can be only partially effective for the drug extraction from the cyclodextrins cavity.

Acknowledgements

The authors wish to thank the University of Athens for having financially supported this work.

References

[1] G. Bettinetti, A. Gazzaniga, P. Mura, F. Giordano, M. Setti, Drug Dev. Ind. Pharm. 18 (1992) 39.
[2] J. Blanco-Mendez, J.L. Vila-Jato, F.J. Otero-Espinar, S. Anguiano-Igea, Drug Dev. Ind. Pharm. 17 (1991) 943.
[3] N. Ganza-Gonzalez, J.L. Vila-Jato, S. Anguiano-Igea, F.J. Otero-Espinar, J. Blanco-Mendez, Int. J. Pharm. 106 (1994) 179.
[4] H.W. Frijlink, A.C. Eissens, A.J.M. Schoonen, C.F. Lerk, Int. J. Pharm. 64 (1990) 195.
[5] F.J. Otero-Espinar, S. Anguiano-Igea, N. Garcia-Gonzalez, J.L. Vila-Jato, J. Blanco-Mendez, Int. J. Pharm. 79 (1992) 149.
[6] N. Sosnowska-Sadlej, L. Kozerski, E. Bednarek, J. Sitkowski, J. Incl. Phenom. Macrocyclic Chem. 37 (2000) 383.
[7] G.N. Valsami, P.E. Macheras, M.A. Koupparis, J. Pharm. Sci. 79 (1990) 1087.
[8] S. Rozou, E. Antoniadou-Vyza, J. Pharm. Biomed. Anal. 18 (1998) 89.
[9] S. Rozou, A. Voulgari, E. Antoniadou-Vyza, Eur. J. Pharm. Sci., 2004, in press.
[10] V. Zecchi, I. Orienti, A. Fini, Pharm. Acta. Helv. 63 (1988) 299.
[11] G. Bettinetti, F. Melani, P. Mura, R. Monnanni, F. Giordano, J. Pharm. Sci. 80 (1991) 1162.
[12] S. Rozou, E. Antoniadou-Vyza, Chromatographia 57 (2003) S81.
[13] A. Bielejewska, K. Duszczyk, A. Kwaterczak, D. Sybilska, J. Chromatogr. A 977 (2002) 225.
[14] R. Nowakowski, P.J.P. Cardot, A.W. Coleman, E. Villard, G. Guichon, Anal. Chem. 67 (1995) 259.
[15] D. Mentzafos, I.M. Mavridis, M.B. Hursthouse, Acta Cryst. C52 (1996) 1220.
[16] A. Rontoyanni, I.M. Mavridis, Acta Cryst. C52 (1996) 2277.
[17] K. Fujimura, T. Ueda, M. Kitagawa, H. Takayanagi, T. Ando, Anal. Chem. 58 (1986) 2668.
[18] F. Gritti, G. Felix, M.F. Achard, F. Hardouin, Chromatographia 56 (2002) 9.