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Separation of *cis*- and *trans*-isomers of thioxanthene and dibenz[*b*,*e*]oxepin derivatives on calixarene- and resorcinarene-bonded high-performance liquid chromatography stationary phases

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Abstract

The chromatographic behavior of six calix[*n*]arene phases (*n*=4, 6, 8) and one calix[4]resorcinarene phase is described for the separation of *cis*- and *trans*-isomers of three thioxanthene (flupentixol, clopenthixol, chlorprothixene) and one benz[*b*,*e*]oxepin derivative (doxepin). The influences of two different organic modifiers (MeOH, MeCN) for the separation of the isomers on every column are described. Different selectivities of the stationary phases exist as a function of the ring size of the calixarenes and their substitution at the ''upper rim'' with *p-tert*.-butyl groups. Furthermore, the influence of free phenol groups on the resorcinarene phase is discussed. Relations between structural elements of the analytes and the retention behavior on the stationary phases are found. The selectivity of the calixarene and resorcinarene stationary phases is compared with a RP-C_{18} phase containing the same base silica. Advantages of the resorcinarene as well as of the calixarene columns exist for the separation of *cis*- and *trans*-isomers of three compounds dependent from the substitution in position 2 of the thioxanthenes, respectively the kind of the basic side chain of all substances.

Keywords: Stationary phases, LC; Mobile phase composition; Positional isomers; Geometrical isomers; Pharmaceutical analysis; Thioxanthenes; Dibenzoxepins; Calixarenes; Resorcinarenes; Doxepin

1. Introduction

Calixarenes and resorcinarenes are oligocondensates prepared from phenols and aldehydes [1,2]. Besides cyclodextrins and crown ethers these molecules are named third-generation host compounds because of their wide range of possibilities in supramolecular chemistry concerning the synthesis of selective receptor molecules [3].

In recent years, the interest in these molecules in analytical and separation chemistry increased because of their affinity to form reversible complexes with neutral as well as charged molecules [4]. In the same way, calixarenes and resorcinarenes are used as selectors in high-performance liquid chromatography (HPLC) bonded on silica gel. First papers describe the employment of functionalized calixarenes for the separation of metal ions and amino acid ester hydrochlorides [5,6]. Several calixarenes with *p-tert*.-butyl substituents at the ''upper rim'' showed high selectivities for the separation of aromatic positional isomers, polycyclic aromatic hydrocarbons

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(PAHs), nucleosides and nucleobases [7–11]. Other polar calixarenes like calix[6]arene-*p*-sulfonates [12] and calix[4]pyrroles [13] were used to separate mono-substituted phenols and other aromatic regioisomers, respectively anionic nucleotides, oligonucleotides, N-protected amino acids and perfluorinated biphenyls. The first paper comparing also unsubstituted calixarenes of different ring size by separations of, i.e., PAHs, barbituric acid derivatives and xanthines discusses the influences of binary eluents on the retention behavior [14]. Also a chiral modified calixarene is described for the discrimination of racemic ethanol derivatives [15].

In contrast to calixarenes, the employment of resorcinarenes in HPLC bonded on silica gel is hardly investigated. [Calix[4]resorcinarenes are used as dynamic coatings on an RP-C_{18} phase for the separation of aromatic positional isomers and nucleobases [16,17]. Nevertheless, these selectors were not covalently bonded on the silica gel.

As far as we know, there is only one paper dealing with separations of *cis*- and *trans*-isomers by calixarene-bonded stationary phases. Gebauer et al. [18] describe such discriminations of proline-containing dipeptides on *p-tert*.-butyl-calixarene-bonded silica gels. The selectivity of the stationary phases was dependent on the ring size of the calixarenes. Differences in elution patterns were discussed by a host–guest complexation of the analytes with the calixarenes.

The investigated thioxanthene derivatives are important neuroleptic drugs. The *cis*-isomer is physiologically more active than the *trans*-isomer [19]. Therefore a determination of the content of the geometric isomers is necessary. HPLC methods for a separation of thioxanthene isomers are described [20–22].

The benz[*b*,*e*]oxepin derivative doxepin is a member of the tricyclic antidepressants with anti-anxiety anti-histamine properties [23]. The *cis*-isomer is more potent of the two geometric forms [24]. Therefore, investigations concerning stereoselective pharmacokinetics are important [25]. HPLC methods for a separation of the two isomers exist in normal-phase [25,26] and reversed-phase modes [27].

This paper describes the possibilities of the use of six calixarene and one resorcinarene phases to separate geometric isomers of three thioxanthene and one dibenz[*b*,*e*]oxepin derivative. The differences in the structures of the analytes are compared with the structures of the selectors on every column and resulting selectivity. Hence, a relation to possible retention mechanisms on these phases is proposed. Furthermore, influences of the kind of organic modifier were investigated. By comparing the calixarene and the resorcinarene phases with a conventional RP-C_{18} phase, advantages in selectivity on phases with bonded resorcinarenes or calixarenes are demonstrated in certain cases.

2. Conditions

2.1. Chemicals

cis- and *trans*-Isomers of flupentixol, clopenthixol and chloprothixene were obtained from Tropon (Cologne, Germany). *cis*- and *trans*-Doxepin were supplied by Salutas Pharma (Magdeburg, Germany).

HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were purchased from Applichem (Darmstadt, Germany). Water was obtained by bidistillation.

2.2. Equipment

The separations were achieved with a HP1090 II Model equipped with a diode array detection (DAD) system (Hewlett-Packard, Waldbronn, Germany).

2.3. Columns

Caltrex phases (calixarene phases) and the resorcinarene phase were obtained from Synaptec (Greifswald, Germany). All calixarene phases contain silica-bonded calixarenes of different ring size and immobilized via a propyl spacer on Kromasil [Kromasil Si 100, 5 μm, specific surface area/BET: 311 m^2/g, pore volume: 0.9 ml/g, manufacturer: EKA Chemicals (Bohus, Sweden)] by a patented procedure (DE 19602393, EP 0786661 A2 and Wo 97/27479) (Fig. 1). [Caltrex AI – calix[4]arene (0.617 $\mu mol/m^2$; C: 9.72%), Caltrex AII – calix[6]arene (0.350 $\mu mol/m^2$; C: 8.72%), Caltrex AIII – calix[8]arene (0.541 $\mu mol/m^2$; C: 13.82%), Caltrex BI – *para-tert*.-butylcalix[4]arene (0.536

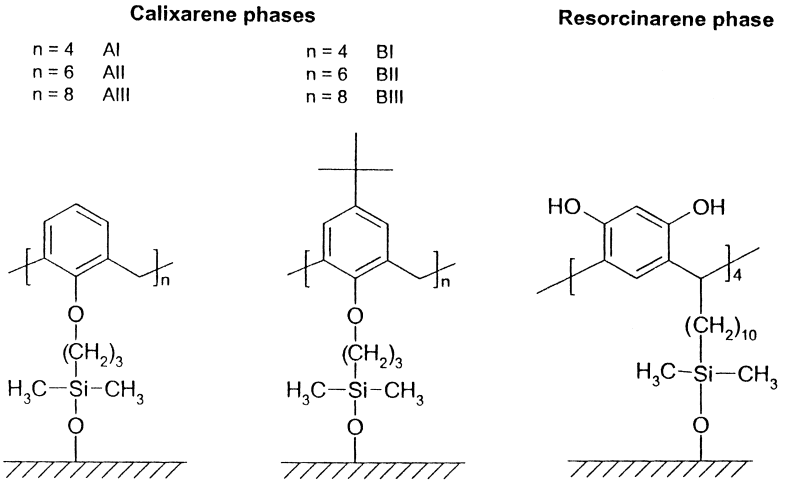

Fig. 1. Structures of the calixarene and resorcinarene stationary phases.

μmol/m^2; C: 10.9%), Caltrex BII – *para-tert.*-butylcalix[6]arene (0.329 μmol/m^2; C: 10.29%), Caltrex BIII – *para-tert.*-butylcalix[8]arene (0.196 μmol/m^2; C: 8.96%)]. The resorcinarene phase (RES; 0.346 μmol/m^2; C: 7.47%) was made by the same procedure like the calixarene phases but resorcinarenes were immobilized via an undecyl spacer on Kromasil.

The Kromasil RP-C_{18} phase (3.5 μmol/m^2; C: 20%) was purchased from CS Chromatography Service (Langerwehe, Germany).

All phases have mean pore diameters of 100 Å, particle diameters of 5 μm and dimensions of 250×4 mm I.D.

2.4. Chromatography

Chromatographic experiments were performed with isocratic elution of binary mobile phases throughout. Binary eluents consisted of buffer in different proportions with MeCN and MeOH. The pH value of the 20 m*M* sodium dihydrogenphosphate buffer was adjusted to 3.5 with H_3PO_4 or NaOH. The eluent was degassed prior to use. UV detection was used at 225 nm. In all cases the column temperature was set at 40°C, the flow-rate was 1 ml/min and injection volumes were 10 μl. Analytes were dissolved in MeOH at a concentration between 0.25 and 0.5 mg/ml. The hold-up times (t_0) were determined from injections of MeOH with UV detection at 220 nm in a MeCN–water (50:50, v/v) mixture as the mobile phase.

3. Results and discussion

3.1. Comparison between calixarene and resorcinarene phases

Four analytes with thioxanthene or dibenz[*b,e*]oxepin basic structures were chosen to study influences of calixarene and resorcinarene phases on the separation of geometric *cis*- and *trans*-isomers. Three thioxanthene derivatives differ in the kind of the basic side chains as well as in the kind of the substituents on position 2. The dibenz[*b,e*]oxepin derivative doxepin has a similar side chain but a different basic structure (Fig. 2).

The selectivity of the calixarene phases for the analytes was investigated as a function of the ring size of the bonded calix[*n*]arenes (n=4, 6, 8) and the substitution at the ''upper rim'' with *p-tert.*-butyl groups. In addition to this, a calix[4]resorcinarene phase was selected to compare selectivities with the other phases.

Two different pH values of the aqueous part of the buffer (pH 2.5 and pH 3.5) were tested. In all cases no significant differences in selectivity were observed. Thus, experiments were performed with a pH value of 3.5 throughout.

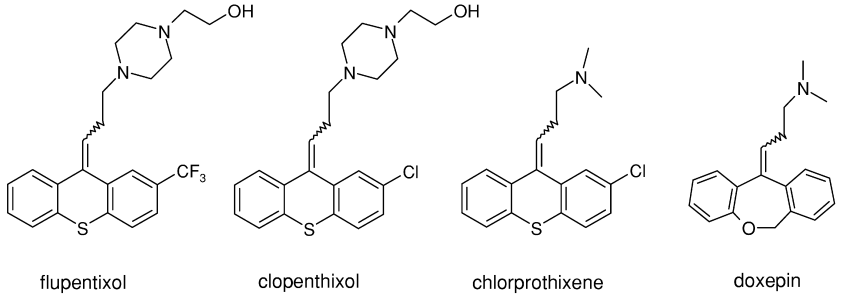

Fig. 2. Structures of all investigated analytes.

The proportions of organic modifier for the separation of every single analyte on all calixarene phases were the same. Although there is a similar carbon content on all calixarene phases between 8.96 and 13.82% the retention times on every column differed in some cases to a bigger extent. This could be due to a different strength of interaction with certain calixarenes. Otherwise, the molar surface coverage with calixarenes varies and is in parts divergent to the carbon content. Therefore, the retention times will not be discussed as a possible marker for the strength of the interactions with calixarenes although it could explain some differences in the selectivities of the columns.

For comparative purposes, the strength of the eluent for the resorcinarene phase was adjusted in such a way that almost the same analysis times concerning calixarene columns were achieved.

3.1.1. HPLC of thioxanthene derivatives

The separation of the geometric *cis*- and *trans*-isomers of flupentixol, clopenthixol and chlorprothixene showed a different behavior on the calixarene and resorcinarene phases. Selectivity of the columns will be discussed as a function of the substitution in position 2 and the kind of the basic side chain. Thus, differences in selectivity of the stationary phases for the three compounds could be due to these differences in the chemical structures. Furthermore, the results give conclusions for retention mechanisms on these phases.

cis-Isomers of the three compounds eluted before the *trans*-isomers. Hence, a stronger interaction between the steric less hindered halogen substituted aromatic of the *trans*-isomers with the host molecules likely occurs.

3.1.1.1. Flupentixol

With a mobile phase containing 30% (v/v) of MeOH no separation of the isomers of flupentixol could be achieved on any calixarene phase (Table 1). In comparison, with an eluent with 55% (v/v) MeCN good selectivities were obtained on phases with bonded calix[4]- and calix[6]arenes (Table 2; Fig. 3). Columns with the largest ring size of the calixarenes were still worse able to separate both geometric isomers. Thus, the ring size of the calixarenes independent from the substitution at the ''upper rim'' has a decisive role for separating these analytes. Small calixarenes are more suitable than larger ones.

Higher separation factors were obtained on columns containing calixarenes that are substituted with *p-tert*.-butyl groups at the ''upper rim''. Thus, in addition to a small ring size calixarene phases of the B series better discriminated the geometric isomers of flupentixol in comparison to that of the A series.

The selectivity of the resorcinarene phase was much better compared to all calixarene phases (Tables 1 and 2; Fig. 3). The bonded resorcinarene has a small ring size with four aromatic units. This corresponds with the advantage of small calix[4]arenes to separate these isomers. Additionally, phenols at the ''upper rim'' of the resorcinarenes are able to interact with the analytes. Possibly, the hydroxyl group of the basic side chain of flupentixol is able to form a bond with the phenols of the resorcinarene. This could be the reason for the very good discrimination of the two isomers on this stationary phase. Both in systems with 55% (v/v) MeOH ($\alpha=1.25$) and 35% (v/v) MeCN ($\alpha=1.16$) flupentixol isomers were completely resolved whereas all calixarene phases of the A and B series showed no selectivity in MeOH systems ($\alpha=1.00$) and a

Table 1
Capacity factors (k') and separation factors (α) of geometric isomers obtained on various columns with MeCN–NaH_2PO_4 buffer (pH 3.5) in different proportions

	AI		AII		AIII		BI		BII		BIII		RES		RP-C_{18}	
	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α
						30:70 (v/v)								35:65 (v/v)		
cis-/*trans*-Flupentixol	12.74		5.13		18.70		13.15		9.00		9.82		5.85		11.37	
	13.29	1.04	5.13	1.00	18.70	1.00	13.99	1.06	9.30	1.03	10.08	1.03	6.77	1.16	11.68	1.03
						30:70 (v/v)								35:65 (v/v)		
cis-/*trans*-Chlorprothixene	8.47		3.38		12.46		7.82		5.86		6.23		5.76		5.75	
	9.27	1.09	3.61	1.07	13.73	1.10	9.07	1.16	6.37	1.09	6.77	1.09	6.10	1.06	6.55	1.14
						30:70 (v/v)								35:65 (v/v)		
cis-/*trans*-Clopenthixol	9.14		3.47		13.54		8.65		6.22		6.53		4.98		6.28	
	9.90	1.08	3.60	1.04	14.46	1.07	9.46	1.09	6.62	1.06	6.98	1.07	5.83	1.17	6.90	1.10
						25:75 (v/v)							20:80 (v/v)		25:75 (v/v)	
trans-/*cis*-Doxepin	5.26		2.56		7.54		5.36		3.97		4.07		8.61		7.80	
	5.26	1.00	2.56	1.00	7.54	1.00	5.36	1.00	3.97	1.00	4.07	1.00	8.61	1.00	7.80	1.00

smaller selectivity with a mobile phase containing MeCN (i.e., Caltrex BI: $\alpha = 1.06$).

3.1.1.2. Clopenthixol

Clopenthixol has the same basic side chain like flupentixol but differs in the substitution in position 2 (Fig. 2). The separation factors for the discrimination of the isomers of this compound on calixarene phases are higher than those for flupentixol on the same phases (Tables 1 and 2). Thus, a more specific interaction of clopenthixol with the cavities of the calixarenes is preferred compared with flupentixol. The kind of the substituent on position 2 of the aromatic system must be responsible for the differences in the selectivity of the columns. Nevertheless, a lot of similarities exist when comparing the

Table 2
Capacity factors (k') and separation factors (α) of geometric isomers obtained on various columns with MeOH–NaH_2PO_4 buffer (pH 3.5) in different proportions

	AI		AII		AIII		BI		BII		BIII		RES		RP-C_{18}	
	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α
							55:45 (v/v)								60:40 (v/v)	
cis-/*trans*-Flupentixol	7.05		1.54		14.44		10.27		4.32		4.80		9.12		9.27	
	7.05	1.00	1.54	1.00	14.44	1.00	10.27	1.00	4.32	1.00	4.80	1.00	11.43	1.25	9.27	1.00
						50:50 (v/v)							55:45 (v/v)		60:40 (v/v)	
cis-/*trans*-Chlorprothixene	9.91		1.40		14.95		7.53		3.86		4.90		11.20		3.67	
	9.91	1.00	1.40	1.00	16.63	1.11	8.63	1.15	4.26	1.10	5.42	1.11	12.24	1.09	4.20	1.15
						50:50 (v/v)							55:45 (v/v)		60:40 (v/v)	
cis-/*trans*-Clopenthixol	10.93		2.04		26.40		14.18		5.94		6.71		8.66		5.84	
	11.59	1.06	2.04	1.00	28.10	1.06	15.74	1.11	6.29	1.06	7.15	1.07	10.40	1.20	6.43	1.10
						35:65 (v/v)								40:60 (v/v)		
trans-/*cis*-Doxepin	9.23		2.13		16.14		9.18		4.70		5.32		6.23		7.49	
	11.54	1.25	2.37	1.11	17.94	1.11	10.91	1.19	5.53	1.18	6.30	1.19	6.23	1.00	8.76	1.17

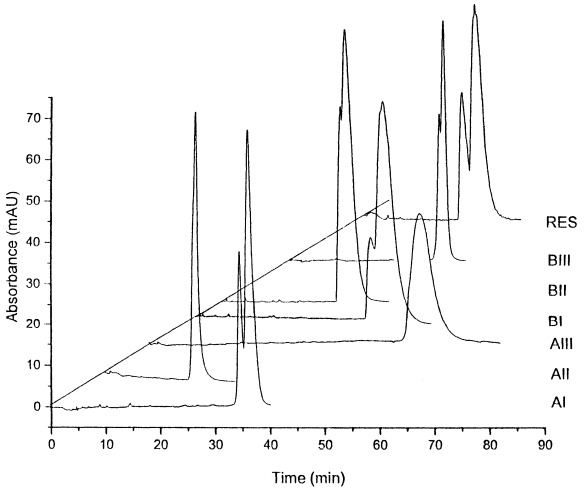

Fig. 3. Comparison of isocratic separations of geometric isomers of flupentixol on six calixarene and one resorcinarene phase. Analytes: *cis*- and *trans*-flupentixol, the *cis*-isomer is eluting first in all cases. Conditions: calixarene phases: MeCN–NaH_2PO_4 buffer (pH 3.5) (30:70, v/v); resorcinarene phase (RES): MeCN–NaH_2PO_4 buffer (pH 3.5) (35:65, v/v).

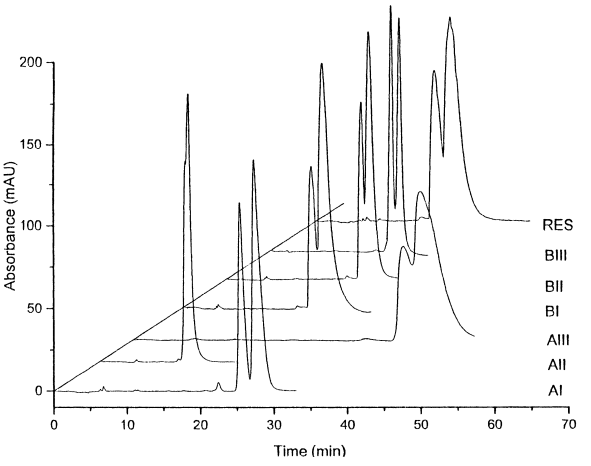

Fig. 4. Comparison of isocratic separations of geometric isomers of clopentixol on six calixarene and one resorcinarene phase. Analytes: *cis*- and *trans*-clopentixol; the *cis*-isomer is eluting first in all cases. Conditions as in Fig. 3.

separations of the two thioxanthene derivatives. For example, eluents with MeCN showed higher separation factors than eluents with MeOH. Nevertheless, in contrast to flupentixol a separation of the isomers is possible in both cases. The differences between the two binary mobile phase systems are especially distinct on Caltrex A phases because a separation of the geometric isomers on Caltrex AII was not possible with MeCN eluents. As for the separation of flupentixol isomers, small calixarenes are preferred for a more specific interaction with clopenthixol with the exception of Caltrex AIII that showed the same selectivity like Caltrex AI. Nevertheless, the resolution on Caltrex AI is much higher than on Caltrex AIII and almost a baseline separation was achieved under these conditions (Fig. 4). Furthermore, columns with calixarenes containing *p-tert.*-butyl groups at the ''upper rim'' have higher separation factors than calixarenes without these groups. This effect is especially pronounced in systems with MeOH.

As for flupentixol, the resorcinarene phase has the best selectivity of all investigated phases to separate the isomers of clopenthixol. In contrast to the calixarene phases, MeOH eluents gave higher separation factors than mobile phases with MeCN (Tables 1 and 2). The small cavity of calix[4]-resorcinarene in combination with the phenolic substituents at the ''upper rim'' seems to be very advantageously for a specific interaction with the resorcinarene stationary phase. The phenols enhance the polarity of the upper part of the cavity. Hence, the hydroxyl group of the side chain could interact with the resorcinarene. In comparison with flupentixol, the separation factors for clopenthixol are smaller. The trifluoromethyl substituent of flupentixol is bigger than the chloro group of clopenthixol. Therefore, the different selectivities for the two compounds have to do with a better inclusion of parts of the *trans*-isomer of flupentixol into the cavity of the resorcinarenes because of the substituent in position 2.

Contrary to calixarene columns, the advantage of the mobile phase system with MeOH could be due to the higher polarity of the resorcinarene phase. Therefore, a better transfer of analytes between stationary phase and eluent could be possible compared with mobile phases containing the more lipophilic MeCN.

The better selectivity of smaller cavities of calixarenes and also of the resorcinarene could indicate that only a little part of the compounds is necessary

for selective contact with the host molecules because the analytes would be too large for a completely complexation. Therefore, a large calixarene is not able to selectively distinguish between both isomers because it is too large and flexible for a preferred inclusion of only the *trans*-isomer.

3.1.1.3. Chlorprothixene

The third investigated thioxanthene derivative was chlorprothixene. Like clopenthixol, it also has a chloro substituent at position 2. The two molecules differ in the basic side chain which is shorter in chlorprothixene and contains no hydroxyethyl substituted piperazinyl group (Fig. 2).

Not many similarities in the selectivities of the calixarene phases were observed for the separations of chlorprothixene isomers compared with separations of clopenthixol isomers on the same columns. While small calixarenes substituted with *p-tert*.-butyl groups have higher separation factors than larger calixarenes, the behavior of phases with calixarenes lacking these groups is contrary to this (Tables 1 and 2). So, a separation of the isomers on Caltrex AI and Caltrex AII with an eluent containing 50% (v/v) MeOH is not possible whereas under the same conditions Caltrex AIII separates the two isomers. Highest selectivities were obtained on phases of the Caltrex B series (Fig. 5). Thus, a deeper cavity of the calixarenes induced by the alkyl substituents is advantageously for a inclusion connected with a selective interaction with these analytes. Altogether, chlorprothixene isomers were most suitable for a separation on calixarene phases compared with the two other thioxanthene derivatives. This could be due to the shorter basic side chain and the substituent in position 2 that allows a better interaction with the hydrophobic cavities of the calixarenes.

Interestingly, stronger differences in the selectivity with different organic modifiers were obtained only on Caltrex A columns. This behavior corresponds with the results observed with clopenthixol. Possibly, this could be due to a different agree of absorption of organic modifier into the stationary phase [14] because calixarenes are known to form complexes also with a lot of small neutral molecules like alcohols [28] and MeCN [29]. The amount of absorbed organic modifier could be dependent from the substitution of the calixarenes. Therefore, stronger differences in selectivities of the stationary phases would be the consequence.

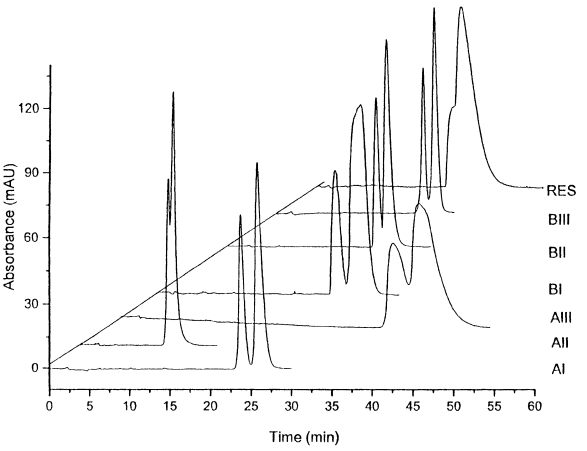

Fig. 5. Comparison of isocratic separations of geometric isomers of chlorprothixene on six calixarene and one resorcinarene phase. Analytes: *cis*- and *trans*-chlorprothixene; the *cis*-isomer is eluting first in all cases. Conditions as in Fig. 3.

The resorcinarene phase shows a very different behavior compared with the separations of the isomers of flupentixol and clopenthixol. Smaller separation factors for the isomers of chlorprothixene were observed on this phase. Thus, even the separations on calixarene phases are better for this compound (Fig. 5). Chlorprothixene does not have a hydroxyl group in the side chain like the other two thioxanthenes. This could be responsible for the different behavior because no interactions between the phenols of the resorcinarene and the alcohol in the side chain of the analyte can occur. Nevertheless, a separation of chlorprothixene isomers with $\alpha = 1.09$ was achievable. Advantages again existed in systems with MeOH compared to systems with MeCN like also observed for separations of the other thioxanthenes. This could be due again with a faster transfer of the analytes between the stationary phase and the eluent because the resorcinarene phase has more polar parts than the calixarene phases.

3.1.2. HPLC of a dibenz[b,e]oxepin derivative

Doxepin is a tricyclic oxepin derivative with the same basic side chain like chlorprothixene. In contrast to the thioxanthenes, geometric isomerism is

caused by the oxepin ring but not by a substitution of the aromatic part (Fig. 2). This could be the reason why the elution order compared with thioxanthenes reversed because *cis*-doxepin elutes after *trans*-doxepin on all calixarene phases. Probably, other interaction mechanisms for this analyte are responsible for this behavior.

As for the separation of flupentixol isomers, *cis*- and *trans*-doxepin could not be separated on any calixarene phase with eluents containing MeCN (Table 1). Nevertheless, by using of mobile phases with 35% (v/v) MeOH the two isomers were resolved and differences in selectivity between the calixarene columns were observed (Table 2; Fig. 6). The ring size of the calixarenes of the A series has an important influence on the separation whereas all Caltrex B phases showed a similar selectivity independent from the size of calixarene cavities. The smallest calix[4]arene on Caltrex AI showed the highest separation factor ($\alpha = 1.25$) for both isomers. On Caltrex B phases this factor was minor ($\alpha = 1.19$ or 1.18) although also good separations were achieved on these phases. Not so good separations compared with the other calixarene phases were obtained on Caltrex AII and Caltrex AIII with $\alpha = 1.11$. Thus, not many similarities exist compared with the separation of chlorprothixene isomers that have the same side chain. Hence, a different retention mechanism must be responsible for this behavior. While the substituent in position 2 of the thioxanthenes obviously interacts with the calixarenes such an interaction in the case of doxepin is not possible. Unsubstituted aromatics on the two sides of the oxepin ring likely interact differently strong with calixarenes according the geometric position of the side chain. Therefore, hydrophobic and $\pi-\pi$ interactions between aromatics probably occur like also described for explanations of other phenomena on these phases [14]. Possibly, the large calixarenes are too flexible for a selective contact with the isomers whereas small calixarenes are much better fitting. The substitution with *p-tert*.-butyl groups at the ''upper rim'' of the calixarenes could hinder interactions only between aromatic systems. Thus, the Caltrex AI had the best selectivities for this compound.

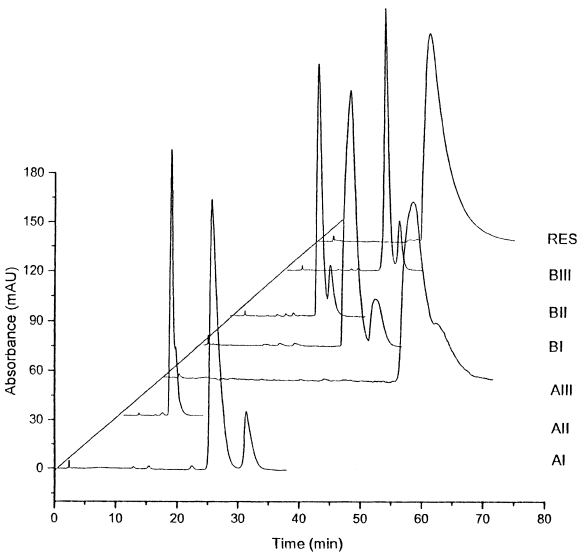

Fig. 6. Comparison of isocratic separations of geometric isomers of doxepin on six calixarene and one resorcinarene phase. Analytes: *trans*- and *cis*-doxepin; the *trans*-isomer is eluting first in all cases. Conditions: calixarene phases: MeOH–NaH_2PO_4 buffer (pH 3.5) (35:65, v/v); resorcinarene phase (RES): MeOH–NaH_2PO_4 buffer (pH 3.5) (40:60, v/v).

The resorcinarene phase was not able to separate *cis*- and *trans*-doxepin both with eluents containing MeOH and MeCN (Tables 1 and 2; Fig. 6). Therefore, the tendency of worse separations of analytes without an hydroxyl group in the side chain (chlorprothixene) is confirmed by doxepin. In addition to this, a substituent on the aromatic part of the analyte seems to be necessary for a separation. Hence, doxepin isomers lacking such halogenated substituents were not resolved on this column. Probably, the benzene rings of this compound are too lipophilic to specifically interact with the resorcinarene that has more polar properties than the calixarenes.

3.2. Comparison between calixarene and resorcinarene phases with a conventional reversed-phase

Because of the wide employment of RP-C_{18} phases for the separation of geometric isomers a comparison with stationary phases containing bonded calixarenes and resorcinarenes was carried out. The RP-C_{18} phase contains the same base silica (Kromasil). Thus, this factor does not influence discussed selectivity differences. Otherwise, the carbon content and the surface coverage is higher than

on the other stationary phases. Therefore, stronger retention of the analytes and different accessability of silanol groups could contribute to a different selectivity profile.

For comparative purposes, the strength of the eluent was adjusted in such a way that almost similar analysis times on the RP-C_{18} were achieved.

The resorcinarene phase was very selective for the separation of the isomers of the thioxanthenes with a side chain containing a hydroxyethyl substituted piperazinyl group. This was due to an interaction between the hydroxyl group of the analyte and phenol groups of the resorcinarene. Such interactions are not given on calixarene phases. Consequently, smaller separation factors were obtained. Also the RP-C_{18} phase is not able to form such bonds. Therefore the selectivity of this phase was not as high for flupentixol (Fig. 7) as well as for clopenthixol compared to the resorcinarene phase. Nevertheless, the separation factor for the discrimination of the clopenthixol isomers on the RP-C_{18} column (α= 1.10) is in a similar range like those obtained on calixarene phases (Caltrex BI: α=1.09).

For the separation of chlorprothixene Caltrex BI was most appropriate of all calixarene and resorcinarene phases. The RP-C_{18} phase shows similar separation factors (Tables 1 and 2). Thus, no real advantage of Caltrex phases in the selectivity for the separation of these two isomers was observed. Furthermore, the resolution on the RP-C_{18} phase is higher (Fig. 8). That could be explained with better kinetic properties in the process of partition between mobile and stationary phase. Possibly, the inclusion of parts of the analytes into the cavities of the calixarenes needs a longer time than the interaction of analytes with the hydrocarbon chains of the RP-C_{18} phase. The selectivity of the resorcinarene phase was much poorer for this compound.

Doxepin isomers were very good separated on calixarene phases with eluents containing MeOH. Caltrex AI showed the highest separation factor (α= 1.25) of all columns. The RP-C_{18} phase showed a minor selectivity (α=1.17) for this compound although most other calixarene phases had similar separation factors (Table 2). Thus, a discrimination of doxepin isomers is advantageously on calixarene phases with bonded calix[4]arenes compared with the RP-C_{18} material (Fig. 9). Stationary phases containing small calixarenes without *p-tert.*-butyl groups interact selective with this compound. That could be due to a better interaction and a good fitting of aromatic parts of the analytes and the aromatic units of these calixarenes. The resorcinarene phase showed no selectivity neither with MeCN nor with MeOH eluents (Tables 1 and 2). Probably, the stationary phase is too polar for a complexation of

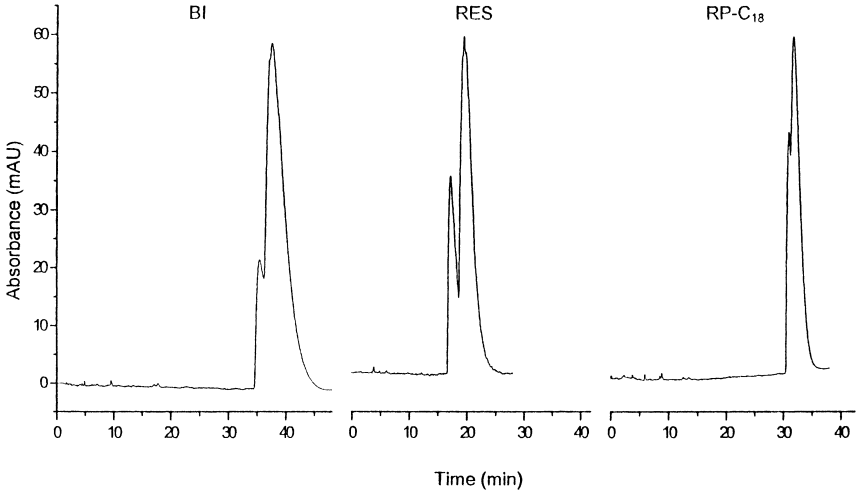

Fig. 7. Comparison of isocratic separations of geometric isomers of flupentixol on Caltrex BI, the resorcinarene phase (RES) and the RP-C_{18} phase. Analytes: *cis*- and *trans*-flupentixol; the *cis*-isomer is eluting first in all cases. Conditions: calixarene phase: MeCN–NaH_2PO_4 buffer (pH 3.5) (30:70, v/v); resorcinarene phase (RES) and RP-C_{18} phase: MeCN–NaH_2PO_4 buffer (pH 3.5) (35:65, v/v).

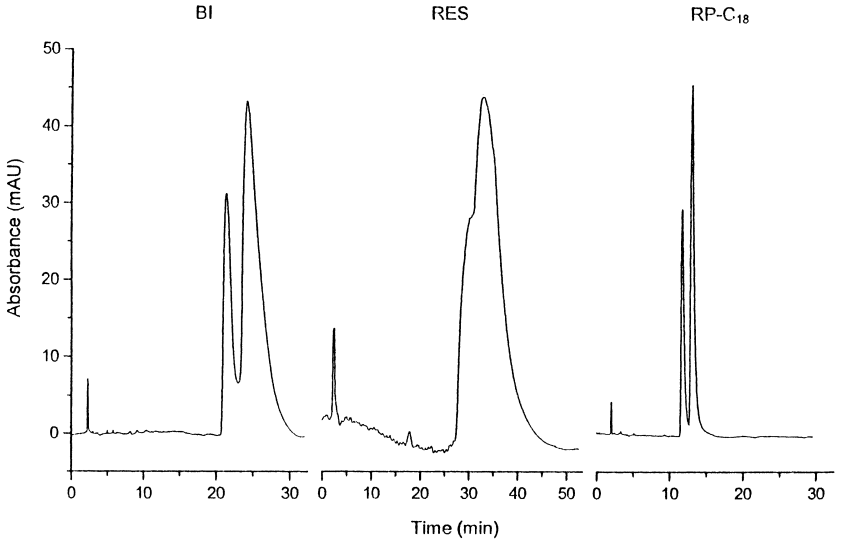

Fig. 8. Comparison of isocratic separations of geometric isomers of chlorprothixene on Caltrex BI, the resorcinarene phase (RES) and the RP-C_{18} phase. Analytes: *cis*- and *trans*-chlorprothixene; the *cis*-isomer is eluting first in all cases. Conditions: calixarene phase: MeOH–NaH_2PO_4 buffer (pH 3.5) (50:50, v/v); resorcinarene phase (RES): MeOH–NaH_2PO_4 buffer (pH 3.5) (55:45, v/v); RP-C_{18} phase: MeOH–NaH_2PO_4 buffer (pH 3.5) (60:40, v/v).

moieties of doxepin into the cavities of the resorcinarenes.

Work is in progress to study the retention behavior of more *cis*- and *trans*-isomers on these phases to clarify which mechanisms are responsible for the selectivity.

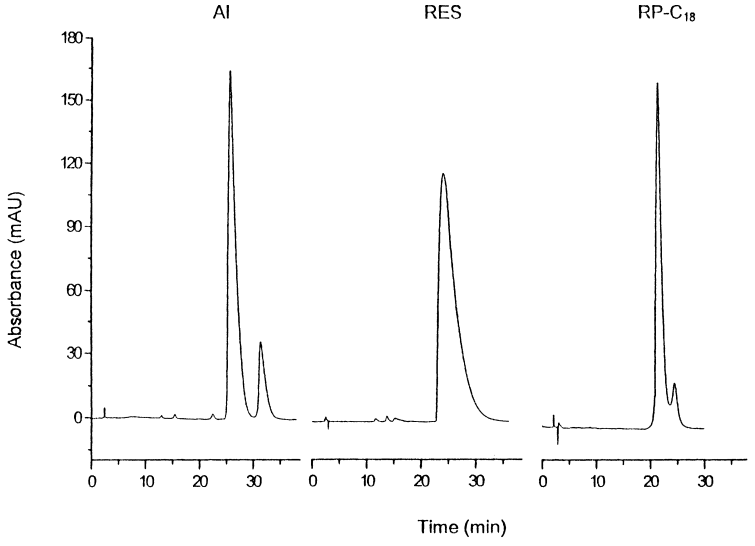

Fig. 9. Comparison of isocratic separations of geometric isomers of doxepin on Caltrex AI, the resorcinarene phase (RES) and the RP-C_{18} phase. Analytes: *trans*- and *cis*-doxepin; the *trans*-isomer is eluting first in all cases. Conditions: calixarene phase: MeOH–NaH_2PO_4 buffer (pH 3.5) (35:65, v/v); resorcinarene phase (RES) and RP-C_{18} phase: MeOH–NaH_2PO_4 buffer (pH 3.5) (40:60, v/v).

4. Conclusions

The results show that silica gels containing covalently bonded calix[*n*]arenes or calix[4]resorcinarene have a different selectivity for the separation of *cis*- and *trans*-isomers of thioxanthene and dibenz[*b*,*e*]oxepin derivatives.

The resorcinarene phase is able to interact specifically with the isomers of flupentixol and clopenthixol that possess polar side chains with a hydroxyl group. An interaction of this part with phenols of the bonded resorcinarenes could explain the best selectivities of all investigated columns.

Chlorprothixene and doxepin isomers were most best discriminated on calixarene phases with small calixarenes. A selective interaction of these more lipophilic compounds with the hydrophobic cavities of the calixarenes is obviously preferred.

In most cases, eluents with MeCN gave higher separation factors on calixarene phases whereas MeOH containing mobile phases were advantageously for separations on the resorcinarene column.

In comparison with an RP-C_{18} phase, advantages of the resorcinarene phase for the separation of the isomers of flupentixol and clopenthixol and for the Caltrex AI phase for the isomers of doxepin were observed although the resolution on the RP-C_{18} phase was better. For chlorprothixene similar selectivities on calixarene phases as well as on the RP-C_{18} phase were obtained.

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References

[1] C.D. Gutsche, Calixarenes, Monographs in Supramolecular Chemistry, Royal Society of Chemistry, Cambridge, 1989.
[2] V. Böhmer, Angew. Chem. 107 (1995) 785.
[3] S. Shinkai, Tetrahedron 49 (1993) 8933.
[4] R. Ludwig, Fresenius J. Anal. Chem. 367 (2000) 103.
[5] J.D. Glennon, E. Horne, K. O'Connor, G. Kearney, J.S. Harris, M.A. McKervey, Anal. Proc. Incl. Anal. Commun. 31 (1994) 33.
[6] J.D. Glennon, E. Horne, K. Hall, D. Cocker, A. Kuhn, S.J. Harris, M.A. McKervey, J. Chromatogr. A 731 (1996) 47.
[7] S. Friebe, S. Gebauer, G.J. Krauss, G. Goermar, J. Krueger, J. Chromatogr. Sci. 33 (1995) 281.
[8] S. Gebauer, S. Friebe, G. Gübitz, G.J. Krauss, J. Chromatogr. Sci. 38 (1998) 383.
[9] W. Xu, J.-S. Li, Y.-Q. Feng, S.-L. Da, Y.-Y. Chen, X.-Z. Xiao, Chromatographia 48 (1998) 245.
[10] X.-Z. Xiao, Y.-Q. Feng, S.-L. Da, Y. Zhang, Chromatographia 49 (1999) 643.
[11] T. Sokoließ, D. Keßler, U. Menyes, U. Roth, Th. Jira, Laborpraxis 7/8 (2000) 70.
[12] Y.K. Lee, Y.K. Ryu, J.W. Ryu, B.E. Kim, J.H. Park, Chromatographia 46 (1997) 507.
[13] J.L. Sessler, P.A. Gale, J.W. Genge, Chem. Eur. J. 4 (1998) 1095.
[14] T. Sokoließ, U. Menyes, U. Roth, Th. Jira, J. Chromatogr. A 898 (2000) 35.
[15] L.O. Healy, M.M. McEnery, D.G. McCarthy, S.J. Harris, J.D. Glennon, Anal. Lett. 31 (1998) 1543.
[16] M. Pietraszkiewicz, O. Pietraszkiewicz, M. Kozbial, Polish J. Chem. 72 (1998) 1963.
[17] O. Pietraszkiewicz, M. Pietraszkiewicz, Polish J. Chem. 72 (1998) 2418.
[18] S. Gebauer, S. Friebe, G. Scherer, G. Gübitz, G.-J. Krauss, J. Chromatogr. Sci. 36 (1998) 388.
[19] S. Tammilehto, M. Sysmäläinen, P. Mäkinen, J. Chromatogr. 285 (1984) 235.
[20] A. Li Wan Po, W.J. Irwin, J. Pharm. Pharmacol. 31 (1979) 512.
[21] T. Aaes-Jørgensen, J. Chromatogr. 183 (1980) 239.
[22] A. Tracqui, P. Kintz, V. Cirimele, F. Berthault, P. Mangin, B. Ludes, J. Anal. Toxicol. 21 (1997) 314.
[23] D. Badenhorst, F.C.W. Sutherland, A.D. de Jager, T. Scanes, H.K.L. Hundt, K.J. Swart, A.F. Hundt, J. Chromatogr. B 742 (2000) 91.
[24] S. Ross, in: D. Smith (Ed.), Handbook of Stereoisomers: Drugs in Psychopharmacology, CRC Press, Boca Raton, FL, 1984, p. 24.
[25] K.K. Midha, J.W. Hubbard, G. McKay, E.M. Hawes, E.D. Korchinski, T. Gurnsey, J.K. Cooper, R. Schwede, Eur. J. Clin. Pharmacol. 42 (1992) 539.
[26] J. Yan, J.W. Hubbard, G. McKay, K.K. Midha, J. Chromatogr. B 691 (1997) 131.
[27] C. Dilger, Z. Salama, H. Jaeger, Arzneim.-Forsch. 38 (1988) 1525.
[28] K.-E. Bugge, W. Verboom, D.N. Reinhoudt, S. Harkema, Acta Crystallogr., Sect. C 48 (1992) 1848.
[29] M.A. McKervey, G. Seward, G. Ferguson, B.L. Ruhl, J. Org. Chem. 51 (1986) 3581.