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Enantiomeric enrichment of non-racemic antihistamines by achiral high-performance liquid chromatography

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Abstract

Enantiomers of several different antihistamines were shown to undergo enantiomer enrichment (auto-resolution) under achiral conditions. Using an aminopropyl silica gel column with a hexane–isopropanol mobile phase, non-racemic mixtures yielded resolutions of very high optical purity. It has been demonstrated that auto-resolution of antihistamines is concentration-dependent, as very dilute samples are not resolved. The benzylic hydrogen on the chiral carbon appears to play a major role in the chiral recognition process.

Keywords: Auto-resolution; Chiral stationary phases; Enantiomer separation; Antihistamines; Chlorpheniramine

1. Introduction

Until recently, it was generally assumed that to achieve resolution of enantiomers using high-performance liquid chromatography (HPLC), a chiral mobile phase additive with an achiral column or a chiral stationary phase (CSP) with an achiral mobile phase must be used. In addition, a pre-column derivatization of the enantiomers with an optically pure reagent to form diastereomers that can be separated under achiral conditions could also be employed. Each method has its advantages and disadvantages.

Bioactive chiral molecules, for example, certain drugs, can exhibit great differences in the physiological activities of the two enantiomers [1], therefore, resolution of these chiral compounds is important. This difference in physiological activity is observed with various antihistamines, yet, they are usually administered as the racemate. For example, (*S*)-chlorpheniramine provides the beneficial antihistamine activity, while (*R*)-chlorpheniramine is largely responsible for the sedative side effects of this drug [2]. The increased US Food and Drug Administration (FDA) restrictions on the use of racemic drugs has prompted the search for more efficient and less costly methods for resolving enantiomeric mixtures.

In an effort to improve upon existing methods [3–6] of antihistamine resolution by HPLC, we prepared several (*S*)-phenylsuccinic acid based CSPs. While testing these CSPs, we repeatedly encountered partial resolution of non-racemic antihistamine mixtures, irrespective of the CSP used. Upon further investigation, we determined that certain antihistamines undergo auto-resolution, that is, non-racemic mixtures are partially resolved while simultaneously using an achiral mobile and stationary phase. Various examples of this, until recently unknown, resolution method have been reported [7–

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15]. Auto-resolution has been reviewed [16,17] and a computer simulation paper addressing the theoretical aspects of this resolution process has also been published [18]. Auto-resolution has also been referred to as self-induced chiral recognition [10], self-amplification of optical activity [8], or enantiomer-differentiation induced by enantiomeric enrichment [9].

It has been proposed [19] that auto-resolution occurs when associations, such as dimer formation, takes place between the molecules to be resolved. Evidence of differential enantiomeric associations appears to be widespread among optically active compounds, as shown by the self-induced nonequivalence of NMR spectra [20]. Two types of associations have been proposed to occur [19]. Enantiomers of the same configuration can associate together (*RR* or *SS*, homo-dimer), or enantiomers of the opposite configuration can associate together (*RS*, hetero-dimer). These homo- and hetero-dimers are diastereomeric in nature and, given the appropriate conditions, can be used to achieve separation under achiral conditions. If one enantiomer is in excess (a non-racemic mixture), the homo-dimer composed of this excess enantiomer can form to a greater extent than the homo-dimer of the minor enantiomer. Alternatively, the minor enantiomer has a greater chance of forming a hetero-dimer, since there is an excess of the ''other'' enantiomer to associate with it, and resolution of the aforementioned ''diastereomers'' is possible based on differences in their physical properties. With a racemic mixture, although the hetero-dimer (*RS*) can form, both homo-dimers (*RR* and *SS*) are formed in equal amounts, and since they are enantiomers, they cannot be resolved under achiral conditions.

2. Experimental

2.1. High-performance liquid chromatography

The HPLC system used in this study consisted of a Waters 501 HPLC Pump, fitted with a Waters U6K Injector, a 20-μl sample loop and a Waters Lambda-Max Model 481 LC spectrophotometer detector, which was set at a wavelength of 261 nm for all HPLC analyses. Chromatography was carried out at ambient temperatures and all flow-rates were 1 ml/min, unless otherwise indicated. The aminopropyl silica gel columns (5 μm particle size) were purchased from Rainin Instrument (Woburn, MA, USA) and were 25 cm×0.46 cm I.D. The Phenomenex chiral column (Phase 3014, 5 μm particle size) was purchased from Phenomenex (Torrance, CA, USA) and was 25 cm×0.40 cm I.D. Void volumes were determined using 2-propanol.

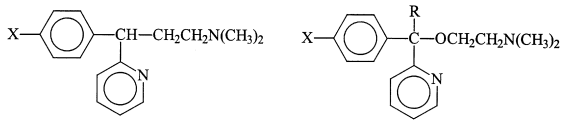
2.2. Chemicals

All solvents used were purchased from Mallinckrodt (Chesterfield, MO, USA) and were HPLC grade. They were deaerated by standard techniques before use. Racemic chlorpheniramine maleate, (*S*)-chlorpheniramine maleate, racemic brompheniramine maleate, (*S*)-brompheniramine maleate, racemic pheniramine maleate, racemic carbinoxamine maleate and racemic doxylamine succinate were purchased from Sigma (St. Louis, MO, USA).

All antihistamines were used as the free base and their structures are shown in Fig. 1. The free base was obtained by stirring the appropriate salt with 20% potassium carbonate for 10 min. The aqueous mixture was extracted twice with diethyl ether, dried over sodium sulfate, and the ether removed under reduced pressure. The yield of antihistamine free base, as an oil, was 95 to 97% and was used without further purification.

2.3. Non-racemic mixtures

Non-racemic chlorpheniramine (**1**) and brompheniramine (**2**) were obtained by mixing the appropriate amounts of racemic and the corresponding optically pure enantiomer. Optically pure (*R*)-chlorpheniramine (**1**) was obtained by a published res-



Chlorpheniramine (**1**) X = Cl
Brompheniramine (**2**) X = Br
Pheniramine (**3**) X = H

Carbinoxamine (**4**) X = Cl, R = H
Doxylamine (**5**) X = H, R = CH_3

Fig. 1. Structure of antihistamines. (**1**) Chlorpheniramine, X=Cl; (**2**) brompheniramine, X=Br; (**3**) pheniramine, X=H; (**4**) carbinoxamine, X=Cl, R=H and (**5**) doxylamine, X=H, R=CH_3.

olution procedure [21] and then mixed with the appropriate amounts of racemic antihistamine to obtain the desired non-racemic mixture. Racemic pheniramine (**3**), carbinoxamine (**4**) and doxylamine (**5**) were injected into a Phenomenex 3014 chiral column. The mobile phase was hexane–ethanol–trifluoroacetic acid (200:18:0.6). Two fractions, corresponding to the elution of the two enantiomers, were collected. This collection process was repeated five times for each of the three antihistamines, combining all of the first fractions and all of the second fractions. Combined fractions I and II were then reinjected and indicated optical purities of greater than 95% (pheniramine), 90% (carbinoxamine) and 95% (doxylamine) for both the (*R*)- and (*S*)-enantiomer of each antihistamine. These nearly optically pure enantiomers were then mixed with the corresponding racemic antihistamine to obtain a non-racemic mixture.

3. Results and discussion

Based on the report [21] that (*S*)-(+)-phenylsuccinic acid [(+)-PSA] was a very good resolving agent for manual resolution of chlorpheniramine (**1**), it was thought that by covalently linking (+)-PSA or various derivatives of it to an aminopropyl silica column, a suitable automated HPLC resolution system could be developed. Thus, in addition to (+)-PSA, *N*-substituted-3-phenylsuccinamic acids [(+)-3-PSAs] were prepared and attached to an aminopropyl silica column. The (+)-3-PSAs prepared were, *N*-propyl-, *N*-phenyl-, (*R*)-*N*-1-phenylethyl- and *N*-propyl-3-(*p*-nitrophenyl)-succinamic acid. While testing the resolving power of these various CSPs, the same partial resolution of non-racemic mixtures of several antihistamines was observed. However, no resolution was observed when racemic samples were tested. Upon further investigation, it was determined that the antihistamines were undergoing auto-resolution, that is, resolution with no other chiral agent present.

Indeed, employing a simple unmodified aminopropyl silica gel stationary phase and a mobile phase consisting of hexane with isopropyl alcohol, several other non-racemic antihistamines were similarly partially resolved by auto-resolution.

A typical example was the partial resolution of non-racemic chlorpheniramine (**1**), enantiomeric excess (ee) 36% of the (*R*)-enantiomer, shown in Fig. 2. Other antihistamines that gave similar results were pheniramine (**3**), carbinoxamine (**4**) and brompheniramine (**2**).

All of these auto-resolvable antihistamines have a doubly benzylic hydrogen. Non-racemic mixtures of doxylamine (**5**), which has a methyl group in place of the benzylic hydrogen, were also tested, but no enantiomeric enrichment was observed. Thus, it appears that the benzylic hydrogen plays an important role in the auto-resolution process.

The concentration limits of the auto-resolution of non-racemic chlorpheniramine (**1**) on an aminopropyl silica gel column was investigated and the results are shown in Table 1. It was observed that the retention factor (k) decreased with increasing concentration of these antihistamines and that the separation factor (α) values passed through a maximum. This suggests that the auto-resolution process is concentration-dependent, thus supporting the idea that auto-resolution is the result of associations, such as dimers, between the (*R*)- and (*S*)-enantiomers. Injection of 5 μl of a solution with a concentration of 1.89 mg/ml onto an aminopropyl column was sufficiently dilute that resolution did not occur. In this case, enantiomers are separated by solvent and dimer formation is not likely. This allows the monomers to associate more strongly with the column, hence the longer retention factors. However, concentrations of

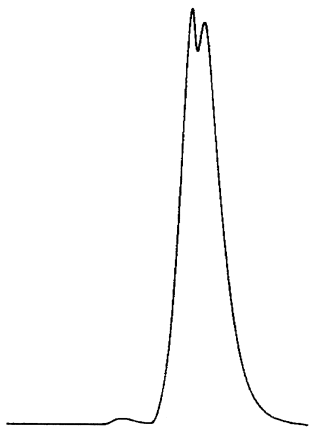


Fig. 2. Chromatogram of partial resolution of non-racemic chlorpheniramine. An aminopropyl silica column was used (for conditions see Section 2.1).

Table 1
Concentration limits of auto-resolution of non-racemic chlorpheniramine (**1**) [ee 36% (*R*) enantiomer]

Volume (μl)	Concentration (mg/ml)	Amount (mg)	k_1	k_2	α
5	1.89	9.5	5.84	[a]	1.00
20	1.89	37.8	4.87	4.93	1.01
20	5.90	118.0	3.21	3.34	1.04
20	19.90	398.0	2.43	2.52	1.04
5	52.60	263.0	2.61	2.71	1.04
10	52.60	526.0	2.17	2.24	1.03
20	52.60	1052.0	1.82	[a]	1.00

[a] No resolution observed.
An aminopropyl silica column was used with a mobile phase of hexane–2-propanol (80:20).
k_1 and k_2 are the retention factors for the first and second eluted peaks, respectively; α=chromatographic separation factor.

non-racemic chlorpheniramine (**1**) as high as 52.6 mg/ml (over 0.5 mg per injection), were partially resolved (k_1=2.17 and k_2=2.24). Higher concentrations resulted in column overload and the loss of detectable resolution. The concentration-dependence of this auto-resolution process could lend itself to larger scale resolutions.

The composition of the mobile phase and hydrogen-bonding are also important in the achievement of partial resolution. Table 2 shows those mobile phases consisting of ethanol (1%), 2-propanol (1%, 5% or 20%), or *tert*.-butanol (20%) with hexane effected partial resolution, whereas higher concentrations of alcohol resulted in a loss of resolution. Therefore, the mobile phase must be sufficiently nonpolar to promote dimer formation, yet polar enough so that the antihistamine can be eluted form the column.

The fact that resolution was not achieved when chloroform or tetrahydrofuran was substituted for the alcohol, suggests that a hydrogen-bonding component of the mobile phase is important. When small amounts of base, such as triethylamine, or acid, like trifluoroacetic acid were added to the mobile phase, resolution was also lost. The presence of acid or base in the mobile phase would be expected to strongly inhibit the hydrogen bond interactions required for dimer formation.

The enantiomeric excess (ee) of the non-racemic mixture was also important to achieve observable partial resolution. Table 3 shows how varying the enantiomeric excess of chlorpheniramine can effect the chromatographic separation factor (α). Racemic chlorpheniramine (**1**) and pure (*S*)-enantiomer eluted as a single peak, while non-racemic mixtures that contained ee 48% and 34% of the (*S*)-enantiomer exhibited two overlapping peaks with separation factors shown. Non-racemic mixtures containing higher than ee 50% or less than 32%, eluted as a single peak at a flow-rate of 1 ml/min and observable resolution was lost. However, a non-racemic mixture containing ee 16% (*S*)-enantiomer did show

Table 2
Resolution of chlorpheniramine (**1**) with mobile phases of hexane containing various solvents

Mobile phase	k_1	k_2
Hexane (100%)	[a]	
2-Propanol (20%)	1.97	2.04
2-Propanol (5%)	3.15	3.27
2-Propanol (1%)	12.26	13.02
tert.-Butyl alcohol (20%)	2.88	3.08
Chloroform (50%)	3.23	[b]
Chloroform (20%)	7.79	[b]
Chloroform (10%)	16.50	[b]
Tetrahydrofuran (10%)	2.00	[b]
Tetrahydrofuran (2%)	3.74	[b]

[a] Did not elute.
[b] No resolution observed.
All injections contained ee 36% (*R*)-chlorpheniramine (**1**).

Table 3
Varying the enantiomeric excess (ee) of chlorpheniramine (**1**) injected

ee	α
100	1.00
86	1.00
48	1.06
34	1.03
16	1.00
0	1.00

All injections were 46 μg; α=chromatographic separation factor.

partial resolution when the flow-rate was reduced to 0.2 ml/min.

Theoretically, as long as there is any difference in the enantiomeric ratio and dimers can form, auto-resolution is possible. This auto-resolution process could be used in conjunction with a partially successful chiral synthesis to obtain an optically purer product.

In order to determine possible differences in retention factors for homo- and hetero-dimers, separately injected equal amounts of racemic and optically pure (*S*)-(+)-chlorpheniramine (**1**) at different column loadings were compared in Table 4. The retention factors reported for each of the column loadings were used to determine which would have the greater retention factor (the pure enantiomer or the racemate). The results indicate that racemic chlorpheniramine (**1**) was retained longer on the aminopropyl silica column than the single enantiomer.

For example, when the sample size was 4.50 mg, a difference in the retention factor (k) of 0.21 was observed between racemic and (+)-chlorpheniramine (**1**); the racemic chlorpheniramine (**1**) being retained longer. This implies that the hetero-dimer, which can form in racemic chlorpheniramine (**1**), interacts more strongly with the stationary phase than the homo-dimer which was solely formed in the optically pure chlorpheniramine case. Hence, in the chromatogram of partially resolved non-racemic chlorpheniramine (**1**), and other structurally related antihistamines which are resolved by auto-resolution, one might expect the first peak to be enriched in homo-dimer (that is, in the more abundant enantiomer).

Table 4
Retention factors of racemic vs. single enantiomer of chlorpheniramine (**1**)

Amount injected (μg)	$k(+)$	$k(\pm)$	$k(\pm)-k(+)$
0.45	8.05	8.08	0.03
2.25	6.63	6.67	0.04
4.50	6.40	6.61	0.21
22.50	5.14	5.17	0.03
44.50	7.98	4.12	0.14

$k(+)$=Retention factor of the single (*S*)-enantiomer, $k(\pm)$= retention factor of racemic chlorpheniramine (**1**), $k(\pm)-k(+)$= difference of the racemic enantiomer from the (*S*)-enantiomer retention factor.

Table 5
Auto-resolution of non-racemic chlorpheniramine (**1**) [ee 36% (*R*)]

Fraction	Time	ee (*R*)-enantiomer
1	15.00–15.20	90.4
2	15.20–15.40	98.4
3	15.40–15.60	92.4
4	15.60–15.80	51.0
5	15.80–16.00	25.0
6	16.00–16.20	20.0
11	17.00–17.20	18.8
19	18.60–18.80	18.2
24	19.60–19.80	18.0

An aminopropyl silica gel column with a mobile phase of hexane–2-propanol (80:20) was used. Flow-rate=0.4 ml/min, concentration injected: 5 μl of 26.3 mg/ml solution. k_1=4.17, k_2=4.25; fractions collected every 0.2 min (12 s) from 15 min.

Based on this assumption, the auto-resolution process was used to resolve non-racemic chlorpheniramine and fractions were collected every 0.20 min (12 s), as components began to elute. These fractions were analyzed using the Phenomenex 3014 chiral column to determine the amount of each enantiomer present.

These results are reported in Tables 5 and 6. Table 5 shows the partial resolution of a mixture of chlorpheniramine (**1**), ee 36% (*R*)-enantiomer. Alternatively, Table 6 shows the fractions collected from the partial resolution of a mixture of chlorpheniramine (**1**), ee 50% (*S*)-enantiomer. Regardless of the enantiomer in excess, it was the one eluted earlier, and was optically purer in the first few fractions.

Table 6
Auto-resolution of non-racemic chlorpheniramine (**1**) [ee 75% (*S*)]

Fraction	Time	ee (*S*)-enantiomer
1	7.60–7.80	74.8
2	7.80–8.00	88.0
3	8.00–8.20	52.8
4	8.20–8.40	19.0
5	8.40–8.60	16.6
6	8.60–8.80	12.4
7	8.80–9.00	4.8
8	9.00–9.20	4.8
9	9.20–9.40	2.8

An aminopropyl silica gel column with a mobile phase of hexane–2-propanol (80:20) was used. Concentration injected: 20 μl of 2.36 mg/ml solution; k_1=1.59, k_2=1.69; fractions collected every 0.2 min (12 s) from 7.60 min.

4. Summary

We have demonstrated that several antihistamines can undergo auto-resolution and that nearly optically pure enantiomer can be obtained. This process of auto-resolution was concentration-dependent, which strongly suggests that associations, such as dimer formation, were responsible for the enantiomeric enrichment of the original non-racemic mixture.

The choice of a hydrogen-bonding mobile phase, such as an alcohol, nonpolar enough to promote these associations, yet sufficiently polar to elute the antihistamine, was necessary. In addition, the benzylic hydrogen also appears necessary for auto-resolution to occur. When this hydrogen was replaced by a methyl group, as in doxylamine, auto-resolution was not observed.

With the growing number of published reports of compounds being resolved under achiral conditions, researchers should be aware that auto-resolution of non-racemic mixtures can occur.

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