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Article

Article

Enantioseparation of Six Antihistamines with Immobilized Cellulose Chiral Stationary Phase by HPLC

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Abstract

A stereoselective high performance liquid chromatography method has been developed for the chiral separation of the enantiomers of six antihistamines, doxylamine, carbinoxamine, dioxopromethazine, oxomemazine, cetirizine and hydroxyzine. The effects of mobile phase additive, column temperature and flow rate on the retention time and resolution were studied. Enantiomeric separation of cetirizine, doxylamine and hydroxyzine were achieved on cellulose tris-(3,5-dichlorophenylcarbamate) immobilized on silica gel chiral stationary phase known as Chiralpak IC ($R_S = 3.74$, $R_S = 1.85$ and $R_S = 1.74$, respectively).

Introduction

The antihistamine drugs, such as carbinoxamine, oxomemazine, dioxopromethazine, doxylamine, cetirizine and hydroxyzine, are used to relieve or prevent the symptoms of hay fever and other allergies (such as allergic rhinitis and atopic dermatitis) (1–3). It is well known that a pair of enantiomers can have different biological activities and toxicological profiles. For example, in the treatment of urticaria, pharmacological activity has been contributed mainly by *R*-cetirizine, while *S*-cetirizine is inactive, so it is necessary to have analytical methodologies for their separation.

Many methods have been reported for the separation of antihistamines, such as capillary electrophoresis (CE), supercritical fluid chromatogram (SFC) and high performance liquid chromatography (HPLC) (4–6). Enantioseparation of dioxopromethazine, cetirizine, hydroxyzine and doxylamine by CE have been widely studied (7–12). In HPLC, Hu *et al.* (13) resolved the enantiomers of cetirizine using HPLC on a chiral ovomucoid column. However, protein chiral stationary phases (CSPs) tend to be less stable. Kang *et al.* (14) separated cetirizine on a Chiralpak AD-H column by LC–MS. In this study, Chiralpak IC, where the chiral selectors is cellulose tris-(3,5-dichlorophenylcarbamate), was used to separate the six antihistamines. Relative to similar columns, it might possess advantages in terms of robustness and the range of mobile phase solvents that can be utilized (15–17).

In this study, the effects of organic modifiers, mobile phase additive, column temperature and flow rate on the retention time and resolution of the enantiomers of oxomemazine, doxylamine, dioxopromethazine, carbinoxamine, hydroxyzine and cetirizine (the structures are shown in Figure 1) were studied.

Experimental

Apparatus

The HPLC instrument used in this study was an Agilent 1,100 series apparatus (Palo Alto, CA, USA) equipped with a quaternary pump, a vacuum degasser, a column oven, a multiple wavelength UV detector, an auto-sampler and HP Chemstation software.

Chemicals

The analytical Chiralpak IC column (250 mm × 4.6 mm, 5 μm) was supplied by Daicel Chemical Industries Ltd (Japan). HPLC-grade *n*-hexane, ethanol and isopropanol were obtained from T&J Kermel Reagent Company. Diethylamine (DEA) was analytically pure and was purchased from T&J Kermel Reagent Company. Doxylamine, cetirizine, carbinoxamine, oxomemazine, dioxopromethazine and hydroxyzine were obtained from New Drugs Research and Development Center of Zhengzhou University.

Sample preparation

Doxylamine, carbinoxamine, oxomemazine, dioxopromethazine, hydroxyzine and cetirizine were dissolved in appropriate amounts of ethanol. The solutions were all filtered (0.22 μm) to prepare the sample solution.

Chromatographic conditions

The basic solvent of mobile phase was *n*-hexane, ethanol or isopropanol was chosen as a mobile phase modifier, and DEA was used as the mobile phase additive. The mobile phase was filtered with a 0.45-μm solvent filter and ultrasonically degassed. The detection wavelengths of cetirizine, carbinoxamine, oxomemazine, hydroxyzine and dioxopromethazine were all set at 227 nm, while doxylamine was set at 262 nm. The volume of sample injected was 10 μL.

Results

Effect of alcohol modifier

The effect that the content of mobile phase modifiers had on the six antihistamines was studied. The results are shown in Table I.

Effect of mobile phase additive DEA

The effect that DEA had on doxylamine, carbinoxamine, dioxopromethazine, cetirizine, oxomemazine and hydroxyzine was studied. The results are shown in Table II.

Effect of the content of DEA

Experiments were carried out to study the effect of content of additive DEA on the enantioseparation of doxylamine, cetirizine and hydroxyzine. The results are shown in Table III.

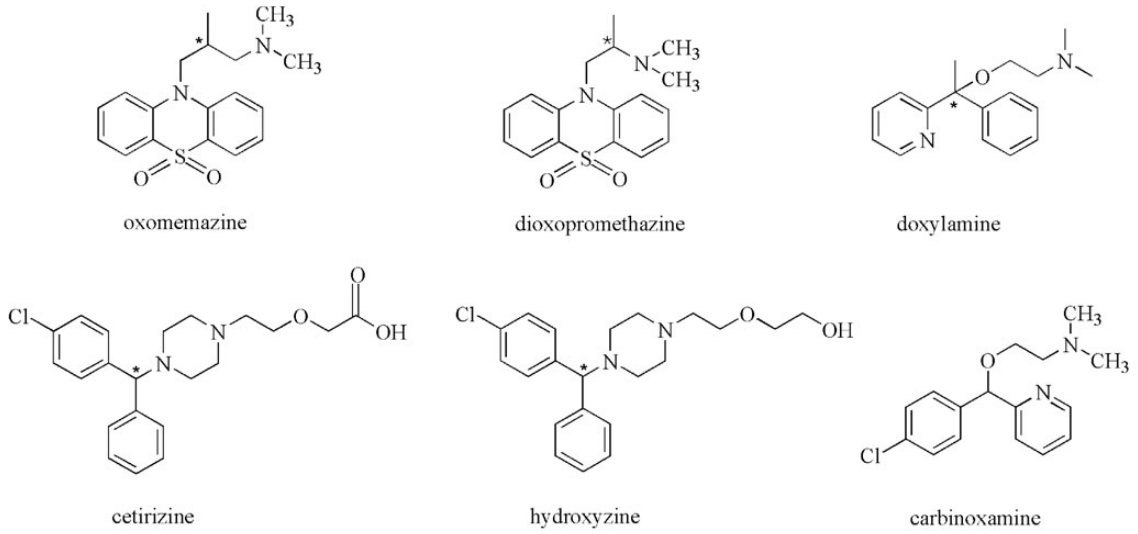

Figure 1. The structures of six antihistamines.

Table I. Effect of Content of Alcohols on the Enantioseparation of Six Antihistamines

Compound	Isopropanol (modifier)					Ethanol (modifier)				
	t_{R1}	t_{R2}	α	R_S	%	t_{R1}	t_{R2}	α	R_S	%
Doxylamine	43.242	48.635	1.13	0.36	5	22.684	25.795	1.16	0.41	5
	23.199		–	–	10	14.547		–	–	10
Carbinoxamine	27.077		–	–	10	14.768		–	–	10
Cetirizine	46.722	56.422	1.22	1.43	5	18.987	21.357	1.15	2.02	5
	25.037	30.295	1.24	1.76	10	12.157	13.383	1.14	1.87	10
	14.513	17.308	1.25	1.88	20	8.666	9.348	1.12	1.59	20
	11.167	13.159	1.25	1.66	30	7.128	7.637	1.13	1.47	30
	10.578	12.254	1.22	1.53	40	6.819	7.213	1.11	1.30	40
Hydroxyzine	38.088	40.725	1.08	0.51	5	22.191		–	–	5
	21.465	22.507	1.06	0.41	10	12.592		–	–	10
	13.274		–	–	20	8.220		–	–	20
Oxomemazine	>100		–	–	10	38.864		–	–	10
Dioxopromethazine	>100		–	–	10	41.684	42.809	1.03	0.37	10
	52.189		–	–	20	19.736	20.144	1.03	0.32	20
	31.660		–	–	30	13.057	13.237	1.02	0.26	30
	22.849		–	–	40	10.183		–	–	40

t_{R1}, t_{R2}: retention times (min); α: separation factor; R_S: resolution factor; "–" means that separation was not seen. *Chromatographic conditions*. The basic solvent of mobile phase was *n*-hexane, the temperature was at 25°C and the flow rate was 0.8 mL min^{-1}.

Table II. Effect of DEA on the Six Antihistamines

Compound	Isopropanol (modifier)					Ethanol (modifier)				
	t_{R1}	t_{R2}	α	R_S	%	t_{R1}	t_{R2}	α	R_S	%
Doxylamine	11.944	12.362	1.05	0.70	5	11.172	12.341	1.15	2.65	5
	9.449		–	–	10	7.465	7.840	1.09	1.85	10
	6.702		–	–	20	6.702	7.011	1.09	1.12	20
	6.008		–	–	30	6.009	6.220	1.07	0.83	30
	5.673		–	–	40	5.418	5.533	1.05	0.56	40
Carbinoxamine	15.724	16.359	1.05	1.24	5	13.437	14.048	1.06	1.22	5
	10.832	11.322	1.06	1.00	10	9.296	9.719	1.07	0.93	10
	7.289	7.534	1.06	0.66	20	7.024	7.219	1.05	0.63	20
	6.342	6.515	1.05	0.59	30	6.154	6.282	1.04	0.53	30
	5.910	6.051	1.05	0.56	40	5.554		–	–	40
Oxomemazine	>100		–	–	5	61.677	63.221	1.03	0.63	5
	>100		–	–	10	31.346	31.769	1.02	0.37	10
	40.385		–	–	20	16.136		–	–	20
Dioxopromethazine	>100		–	–	5	83.919	87.558	1.05	1.20	5
	>100		–	–	10	38.365	39.704	1.04	0.92	10
	43.851	44.670	1.02	0.47	20	18.183	18.668	1.03	0.62	20
	26.733		–	–	30	12.368	12.601	1.03	0.50	30
	19.242		–	–	40	9.451		–	–	40
Cetirizine	32.867	43.704	1.36	5.75	5	18.725	21.306	1.17	2.82	5
	21.355	28.026	1.37	5.08	10	11.985	13.304	1.15	2.59	10
	11.795	14.504	1.31	4.65	20	8.588	9.340	1.14	2.17	20
	9.669	11.570	1.29	4.07	30	7.364	7.910	1.13	1.87	30
	8.625	10.208	1.29	3.74	40	6.424	6.803	1.12	1.56	40
Hydroxyzine	34.758	38.497	1.12	1.99	5	21.507	22.233	1.04	0.66	5
	16.382	17.549	1.09	1.74	10	12.175	12.405	1.03	0.49	10
	9.953	10.471	1.08	1.13	20	8.029		–	–	20
	7.853	8.163	1.07	0.82	30	6.672		–	–	30
	6.942	7.164	1.06	0.64	40	5.796		–	–	40

Chromatographic conditions. The basic solvent of mobile phase was *n*-hexane with 0.1% DEA, and the column temperature was at 25°C with a flow rate of 0.8 mL min^{-1}.

Table III. Effect of Content of DEA on the Enantioselectivity of Three Antihistamines

Compound	t_{R1}	t_{R2}	α	R_S	DEA (%)
Doxylamine	7.465	7.840	1.09	1.85	0.1
	7.447	7.837	1.09	1.93	0.2
	7.302	7.669	1.09	1.88	0.3
Cetirizine	8.625	10.208	1.29	3.74	0.1
	8.401	9.979	1.28	3.66	0.2
	8.391	9.867	1.28	3.63	0.3
Hydroxyzine	16.382	17.549	1.09	1.74	0.1
	16.017	17.116	1.09	1.67	0.2
	15.562	16.575	1.08	1.60	0.3

Chromatographic conditions. Doxylamine: mobile phase was *n*-hexane–ethanol (90/10, v/v); cetirizine: mobile phase was *n*-hexane–isopropanol (60/40, v/v); hydroxyzine: mobile phase was *n*-hexane–isopropanol (90/10, v/v). The column temperature was at 25°C, and the flow rate was 0.8 mL min^{-1}.

Effect of column temperature

The effect that the column temperature had on doxylamine, hydroxyzine and cetirizine was studied. The results are shown in Table IV.

Table IV. Effect of Column Temperature on the Three Antihistamines

Compound	t_{R1}	t_{R2}	α	R_S	Temperature (°C)
Doxylamine	9.019	9.837	1.14	2.44	15
	8.301	8.881	1.11	2.32	20
	7.465	7.840	1.09	1.85	25
	7.156	7.456	1.07	1.65	30
	6.793	7.021	1.05	1.37	35
Cetirizine	10.345	12.519	1.31	3.93	15
	9.388	11.314	1.30	3.85	20
	8.625	10.208	1.29	3.74	25
	7.875	9.095	1.28	3.51	30
	7.353	8.368	1.27	3.20	35
Hydroxyzine	21.379	23.435	1.11	2.10	15
	18.969	20.561	1.10	1.93	20
	16.382	17.549	1.09	1.74	25
	14.925	15.840	1.08	1.56	30
	13.806	14.547	1.07	1.38	35

Chromatographic conditions. Doxylamine: mobile phase was *n*-hexane–ethanol (90/10, v/v, 0.1% DEA); cetirizine: mobile phase was *n*-hexane–isopropanol (60/40, v/v, 0.1% DEA); hydroxyzine: mobile phase was *n*-hexane–isopropanol (90/10, v/v/, 0.1% DEA). The flow rate was 0.8 mL min^{-1}.

Effect of flow rate

The effect that the flow rate had on doxylamine, cetirizine and hydroxyzine was investigated. The results are shown in Table V. The chromatogram of oxomemazine, doxylamine, dioxopromethazine, carbinoxamine, cetirizine and hydroxyzine is shown in Figure 2.

Discussion

Effect of alcohol modifier

The results showed that the enantiomers were not separated well except cetirizine. It was reported that basic or acidic additives in the mobile phase often contribute to improve resolution of chiral compounds (18). Therefore, DEA was used as a mobile phase additive for the six compounds.

Table V. Effect of Flow Rate on the Enantioselectivity of Three Antihistamines

Compound	t_{R1}	t_{R2}	α	R_S	Flow rate (mL min^{-1})
Doxylamine	14.942	15.699	1.09	1.92	0.4
	9.972	10.482	1.09	1.88	0.6
	7.465	7.840	1.09	1.85	0.8
	5.962	6.260	1.09	1.79	1.0
	4.985	5.238	1.09	1.76	1.2
Cetirizine	17.157	20.268	1.29	4.46	0.4
	11.574	13.701	1.29	4.07	0.6
	8.625	10.208	1.29	3.74	0.8
	6.876	6.137	1.28	3.39	1.0
	5.759	6.819	1.29	3.23	1.2
Hydroxyzine	31.543	33.714	1.09	1.91	0.4
	21.566	23.091	1.09	1.83	0.6
	16.382	17.549	1.09	1.74	0.8
	13.042	13.966	1.09	1.66	1.0
	10.952	11.739	1.09	1.59	1.2

Chromatographic conditions. Doxylamine: mobile phase was *n*-hexane–ethanol (90/10, v/v, 0.1% DEA); cetirizine: mobile phase was *n*-hexane–isopropanol (60/40, v/v, 0.1% DEA); hydroxyzine: mobile phase was *n*-hexane–isopropanol (90/10, v/v, 0.1% DEA). The column temperature was at 25°C.

Effect of mobile phase additive DEA

The results showed that the resolution increased significantly, and the retention was decreased with the basic additive DEA, which was due to the interaction between DEA and CSP, which decreased the interaction between the compounds and CSP.

The results also showed that with the increase in the proportion of alcohols, the elution capacity of mobile phase was increased, and the interaction between enantiomers and CSP was decreased, hence the retention time and resolution were decreased.

Effect of the content of DEA

The results indicated that the retention and resolution change little over the DEA concentration range of 0.1–0.3%. Therefore, 0.1% DEA was used.

Effect of column temperature

The parameters are calculated as follows (19):

$$\ln \alpha = -\Delta_{R,S}\Delta H^{\circ}/RT + \Delta_{R,S}\Delta S^{\circ}/R,$$

Here, $\alpha = k'_2/k'_1$, α is separation factor, R is the gas constant and T is the temperature in K, $\Delta_{R,S}\Delta H°$ and $\Delta_{R,S}\Delta S°$ are enthalpy change and entropy change of two enantiomers from the mobile phase to the

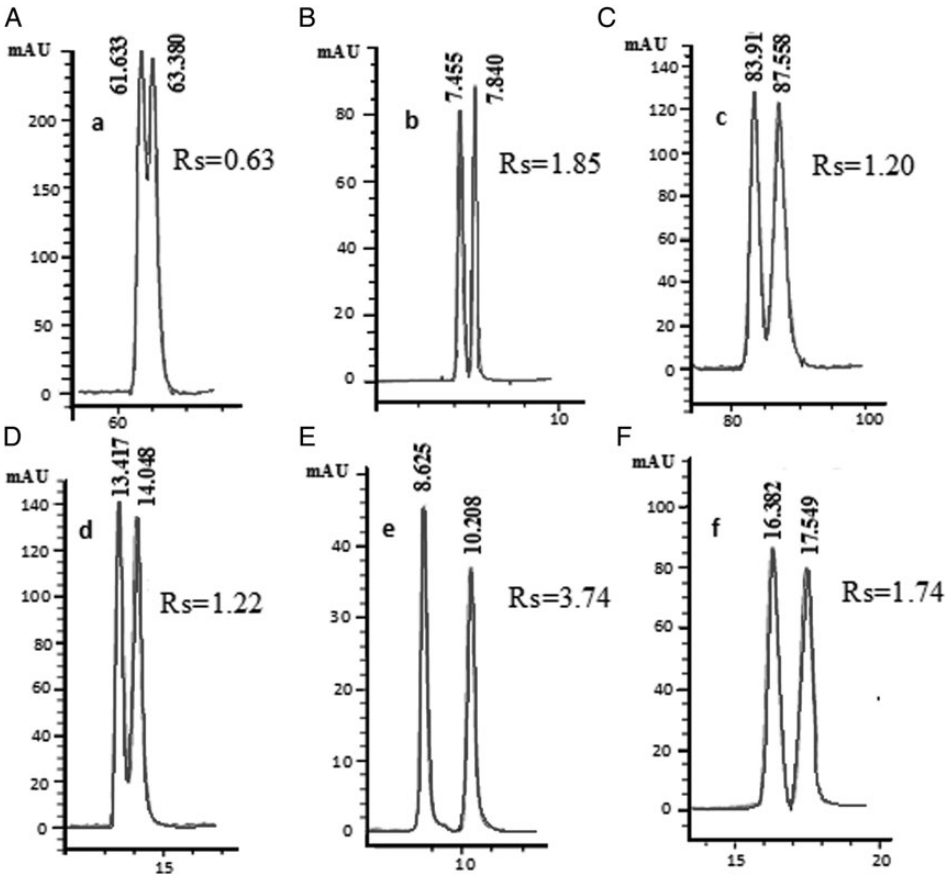

Figure 2. HPLC chromatograms of six antihistamines. (A) oxomemazine, (B) doxylamine, (C) dioxopromethazine, (D) carbinoxamine, (E) cetirizine and (F) hydroxyzine. *Chromatographic conditions*. Oxomemazine, dioxopromethazine and carbinoxamine: *n*-hexane-ethanol (95/5, V/V, 0.1% DEA); doxylamine: *n*-hexane-ethanol (90/10, V/V, 0.1% DEA); cetirizine: *n*-hexane-isopropanol (60/40, V/V, 0.1% DEA); hydroxyzine: *n*-hexane-isopropanol (90/10, V/V, 0.1% DEA). The column temperature was at 25°C, the flow rate was 0.8 mL min^{-1}.

Table VI. The Value of $\Delta_{R,S}\Delta H°$ and $\Delta_{R,S}\Delta S°$ of Three Antihistamines

Compound	Doxylamine	Cetirizine	Hydroxyzine
$\Delta_{R,S}\Delta H°$ (kJ mol^{-1})	−0.365	−0.141	−0.166
$\Delta_{R,S}\Delta S°$ (J mol^{-1})	−1.138	−0.218	−0.473
The regression equation	$\ln \alpha = 365.79/T - 1.1407$ ($r = 0.9978$)	$\ln \alpha = 140.75/T - 0.2180$ ($r = 0.9993$)	$\ln \alpha = 166.55/T - 0.4732$ ($r = 0.9994$)

stationary phase during the distribution process, respectively. Van't Hoff plots were drawn for $\ln \alpha$ vs $1/T$ for two isomers.

Their regression equations are shown in Table VI. The results showed that the linearity of the regression equations was good. From the above equation, the value of $\Delta_{R,S}\Delta H°$ and $\Delta_{R,S}\Delta S°$ was calculated (the results are shown in Table VI). Over the temperature range of 288–308 K, they all conform to $|\Delta_{R,S}\Delta H°| > |T\Delta_{R,S}\Delta S°|$.

Therefore, the chiral separation process of the three compounds was all controlled by enthalpy. As the column temperature increased, a corresponding decrease in retention was observed, the resolution was also decreased. This would be explained by the fact that a thermodynamic process was faster at higher temperatures, which resulted in lower enantiomeric retention.

The results indicated that the column temperature should be carefully controlled for optimum chiral separation of enantiomers. Furthermore, 25°C was closer to room temperature, so the other parameters were optimized at 25°C.

Effect of flow rate

The results showed that the change in separation factor was not significant over the flow rate range of 0.4–1.2 mL min^{-1}. The resolution of doxylamine, hydroxyzine and cetirizine decreased as the flow rate increased over the range of 0.4–1.2 mL min^{-1}. To reduce the analytical time, the flow rate of 0.8 mL min^{-1} was used.

Conclusion

The enantiomers of doxylamine, oxomemazine, carbinoxamine, dioxopromethazine, cetirizine and hydroxyzine were first separated with Chiralpak IC. The optimum chromatographic conditions of six compounds are as follows: cetirizine: *n*-hexane–isopropanol (60/40, v/v, 0.1% DEA), $R_S = 3.74$; hydroxyzine: *n*-hexane–isopropanol (90/10, v/v, 0.1% DEA), $R_S = 1.74$; doxylamine: *n*-hexane–ethanol (90/10, v/v, 0.1% DEA), $R_S = 1.85$; oxomemazine, dioxopromethazine and carbinoxamine: *n*-hexane–ethanol (95/5, v/v, 0.1 DEA), $R_S = 0.63$, $R_S = 1.20$ and $R_S = 1.22$, respectively. The column temperature was at 25°C, and the flow rate was 0.8 mL min^{-1}.

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