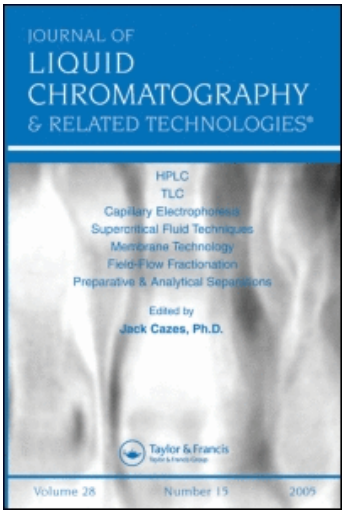

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High-Performance Liquid Chromatographic Separation of 3-[(Cyclopentylhydroxyphenylacetyl)oxy]-1,1-dimethyl-pyrrolidinium Bromide Diastereomers

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF 3-[(CYCLOPENTYLHYDROXYPHENYL-ACETYL)OXY]-1,1-DIMETHYL-PYRROLIDINIUM BROMIDE DIASTEREOMERS

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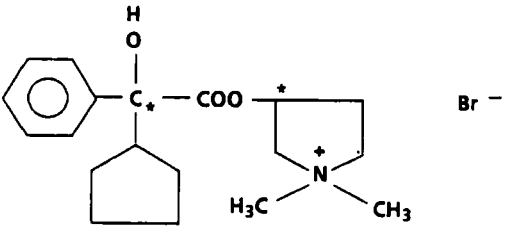
ABSTRACT

Retention of glycopyrrolate and its diastereomeric impurity was studied on a β-cyclodextrin-bonded phase column as function of pH and salt additives. The retention can be modeled by a linear model with pH and ionic strength as independent variables. On the basis of these data, simple and precise method for diastereomeric purity determination of glycopyrrolate was developed.

INTRODUCTION

Glycopyrrolate is a anticholinergic drug marketed under the brand name ROBINUL and is one of the two diastereomeric forms of 3-[(cyclopentylhydroxyphenylacetyl)oxy]-1,1,-dimethylpyrrolidinium bromide (1) I.



I

The two asymmetric centers are marked with asterisks. Four optical isomers exist as two diastereomeric pairs. Glycopyrrolate is represented by the (R,S)-(S,R), pair.

In the synthesis of glycopyrrolate, small amounts of the (R,R)-(S,S)-diastereomer are formed. A simple and reliable procedure for the determination of the diastereomeric purity of glycopyrrolate was desirable.

The ^{13}C-NMR can distinguish between the two diastereomeric forms, and this could be the base of a diastereomeric purity determination method. In practice the detection limit was only 5-10% of the minor diastereomer (2). Attempts to separate the two diastereomers by conventional reversed-phase HPLC or thin-layer chromatography (TLC) remained unsuccessful. We considered that the cyclodextrin-based stationary phases are good candidates to solve our problem. It has been shown in the past by Armstrong et al. (3) that a variety of diastereomers and structural isomers can be separated on cyclodextrin-bonded columns. The key problem of our separation is to reduce the dominant role the quaternary ammonium group has in the chromatographic process. In that respect cyclodextrins appear to be a favorable choice. In a

structurally related series of compounds, namely, n-alkylbenzyl-dimethylammoninum chloride with alkyl chain lengths from 12-18 carbons, selectivity was dominated by the alkyl chain (4).

MATERIALS AND METHODS

Glycopyrrolate was synthetized by the Chemical Resarch Department of A. H. Robins Company, Richmond, VA, USA.

Acetonitrile was spectroscopic grade from E. M. Science, Cherry Hill, NJ, USA. All the other chemicals were reagent grade from Aldrich, Milwaukee, WI, USA.

UV spectra were taken on a Beckman UV5260 spectrometer (Beckman Instruments, Inc., Irvine, CA, USA).

A Varian 5500 HPLC system (Walnut Creek, CA, USA) with variable wavelength UV detector M200 and a 6 port-valve injector with a 10-µL loop was used. Data were processed on a SP4270 Spectra-Physics integrator. We used the column Cyclobond I 25 cm x 4.6 mm I.D., 5-µm particle size from Advanced Separation Technologies, Inc., Whippany, NJ, USA.

The pH was measured with an Orion Research pH meter, Model 611, equipped with a glass electrode.

Statistical data analysis of experimental data was performed with RS1 (BBN) software, run on a DEC-VAX minicomputer and SYSTAT (Systat, Inc.) software, run on a IBM PC-AT, microcomputer.

RESULTS AND DISCUSSION

The separation of the two diastereomers can be achieved on β-cyclodextrin-bonded phase column with mobile phases of high

water content. A mixture of 85 parts water and 15 parts acetonitrile was used in all experiments. The most important factors controlling the separation are pH and salt additive concentrations. The range of pH investigated was from 2.8 to 8.0. A variety of salts have been used as additives to the mobile phase. It appears that there are no specific salt effects. The effect of all the salts used on retention can be related to the ionic strength of the solution.

The retention data of the two diastereomers at different pH values of the mobile phase and different salt additives are listed in Table 1.

The combined effects of pH and ionic strength of the mobile phase on the capacity factors (k') can be described by a linear model. Three-dimensional graphs of k'(A) and k'(B), respectively, versus pH and I are presented in Figures 1 and 2.

The linear models, calculated by multiple linear regression, are

$$k'(A) = -8.650 + 2.127*pH + 50.272*I$$

and

$$k'(B) = -10.121 + 2.494*pH + 57.267*I$$

The pertinent statistics of the two linear models are presented in Table 2.

Neither pH nor the I of the mobile phase significantly changed the separation factor (α). If k' of the less retained diastereomer was higher than 3, the separation factor had a value of 1.13 to 1.19, sufficient for good resolution.

TABLE 1

Retention Data for Glycopyrrolate and Its Diastereomer on β-Cyclodextrin Bonded Phase Column, as Function of pH and Salt Additives

Mobile phase with 85 parts water, 15 parts acetonitrile.

Salt Additive:	C (mole/L)	I(1)	pH	k'A	k'B	α(2)
CH_3COOH	0.0154					
with Et_3N	0.0036	0.010	3.65	0.46	0.52	1.12
	0.0072	0.011	4.15	0.92	1.08	1.18
	0.0108	0.013	4.35	1.48	1.74	1.18
	0.0144	0.015	4.56	1.81	2.12	1.17
	0.0178	0.017	4.97	2.65	3.11	1.17
	0.0123	0.017	5.43	3.57	4.19	1.17
	0.0131	0.017	5.66	4.07	4.79	1.18
	0.0143	0.017	6.00	4.74	5.63	1.19
	0.0167	0.018	6.69	7.03	8.32	1.18
	0.0178	0.018	7.02	8.05	9.45	1.17
	0.0196	0.018	7.52	8.48	10.01	1.18
	0.0216	0.018	8.09	9.80	11.56	1.18
$(NH_4)_2SO_4$	0.025	0.075	5.47	6.91	7.88	1.14
	0.0125	0.038	5.71	6.27	7.12	1.14
	0.0062	0.019	5.99	5.95	6.83	1.15
	0.0031	0.009	6.20	5.26	5.96	1.13
	0.0016	0.005	6.48	4.44	5.00	1.13
	0.0008	0.002	6.37	4.62	5.28	1.15
	0.0004	0.001	6.21	3.19	3.62	1.13
NaCl	0.0125	0.013	6.57	4.28	4.92	1.15
NH_4Cl	0.0125	0.013	5.69	5.32	6.09	1.14
Na_2SO_4	0.0125	0.038	6.11	7.70	8.82	1.14
NaH_2PO_4	0.0125	0.063	4.83	2.84	3.26	1.15

(1)Ionic strength $I = \frac{1}{2} \sum_i (c_i * z_i^2)$

where c = concentration of ion i in mole/L
z = charge of ion i

(2)Separation factor $\alpha = k'_{(B)}/k'_{(A)}$

A - (R,R)-(S,S)-diastereomer
B - glycopyrrolate.

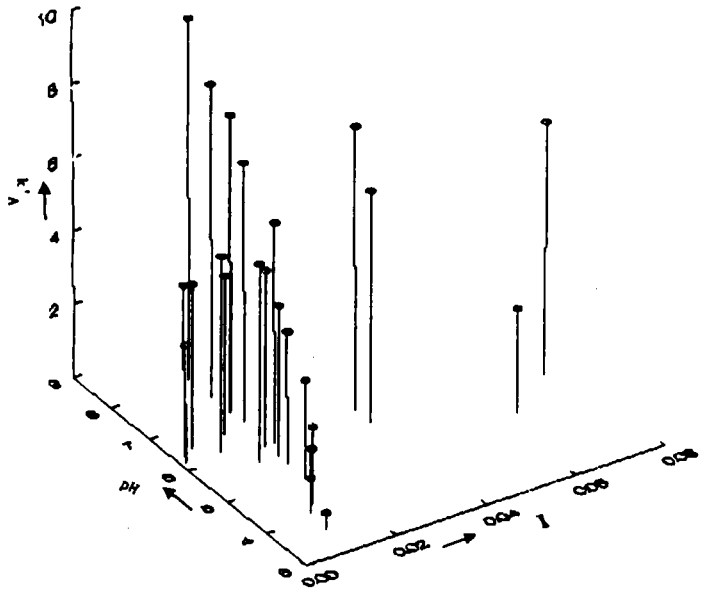


FIGURE 1. Three-dimensional plot of k'_A vs. pH and ionic strength.

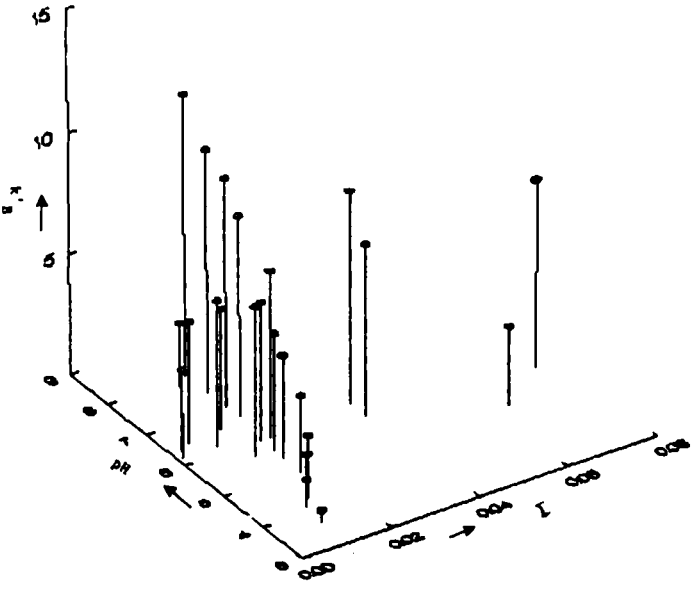


FIGURE 2. Three-dimensional plot of k'_B vs. pH and ionic strength.

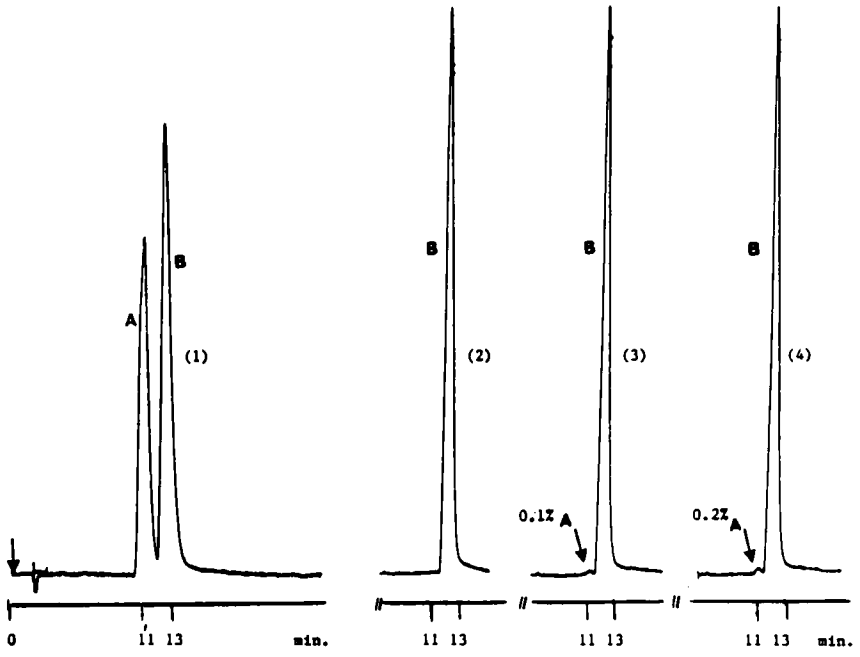


FIGURE 3. Chromatographic separation of glycopyrrolate (B) and its diastereomer(A): 1) mixture of A and B, 2) pure glycopyrrolate, 3) glycopyrrolate spiked with 0.1% w/w diastereomer, and 4) glycopyrrolate spiked with 0.2% w/w diastereomer.

Mobile phase: 0.2 acetic acid in water pH = 7 adjusted with triethylamine - acetonitrile (85:15).
Flow: 1.5 mL/min
Detection: UV 230 nm

Samples 0.5 mg/mL in mobile phase.

The mobile-phase composition chosen for the analysis was 85 parts water, 15 parts acetonitrile, 0.2% acetic acid, and pH adjusted with triethylamine to pH = 7.0. Chromatograms of pure glycopyrrolate and a mixture of glycopyrrolate with its diastereomer are presented in Figure 3. Quantitation of 0.1% w/w of the diasteromeric impurity can be done easily.

TABLE 2

Linear Models, k' vs. pH and I.

Dependent Variable	R (Corr. coeff.)	F (Fisher Ratio)	P (Significance Level)
k'(A)	0.936	71.089	<0.001
k'(B)	0.936	70.451	<0.001

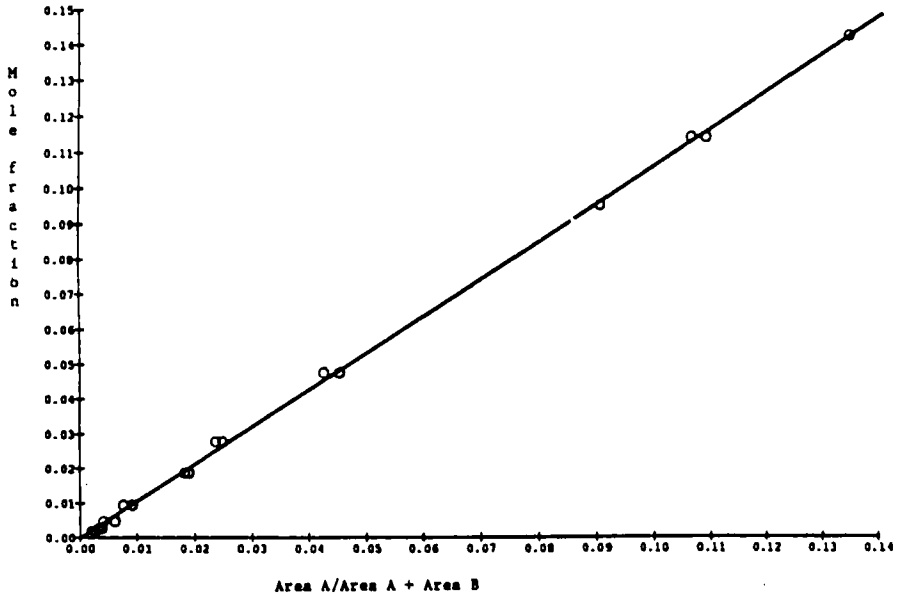


FIGURE 4: Calibration plot for glycopyrrolate and diastereomer.

The two diastereomeric forms have equal extinction coefficients at 258 nm where the adsorptivity is low and impractical to be used for detection. The wavelength chosen for the UV detection was 230 nm with unequal absorptivities of the two diastereomeric forms, hence the necessity of calibration. The calibration graph is presented in Figure 4. The parameters of the calibration line and statistics associated with it are:

%A = 1.065 * [Area(A)/(Area(A) + Area(B))]

F = 54930 (Fisher ratio);

R^2 = 0.999636 (correlation coefficient squared);

p = 1 * 10 exp(-25) (significance level);

n = 21 (data pairs).

In conclusion, the diastereomeric purity of glycopyrrolate was determined in a simple and reliable manner by HPLC on β-cyclodextrin-bonded phase column.

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