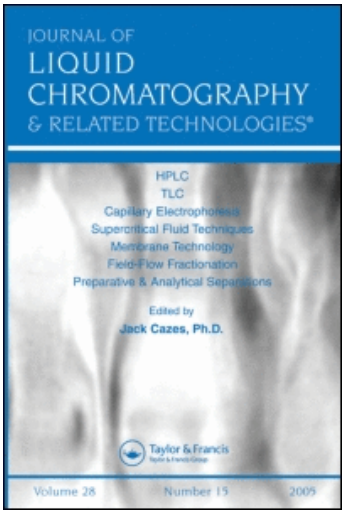

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Irving W. Wainer[a]; Thomas D. Doyle[a]; Christopher D. Breder[a]
[a] Division of Drug Chemistry, Food and Drug Administration, Washington, DC

To cite this Article Wainer, Irving W. , Doyle, Thomas D. and Breder, Christopher D.(1984) 'The Application of HPLC Chiral Stationary Phases to Pharmaceutical Analysis: The Resolution of Some Tropic Acid Derivatives', Journal of Liquid Chromatography & Related Technologies, 7: 4, 731 — 741
To link to this Article: DOI: 10.1080/01483918408073998
URL: http://dx.doi.org/10.1080/01483918408073998

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(4), 731-741 (1984)

THE APPLICATION OF HPLC CHIRAL STATIONARY PHASES TO PHARMACEUTICAL ANALYSIS: THE RESOLUTION OF SOME TROPIC ACID DERIVATIVES

Irving W. Wainer*, Thomas D. Doyle and Christopher D. Breder
Division of Drug Chemistry
Food and Drug Administration
Washington, DC 20204

ABSTRACT

A number of amide and ester derivatives of tropic acid were chromatographed by using a commercially available covalently bonded HPLC chiral stationary phase, (R)-N-(3,5-dinitrobenzoyl)-phenylglycine. The amide derivatives, including pharmacologically important tropicamide, were resolved on this column, but the ester derivatives, including atropine, were not.

INTRODUCTION

Tropic acid, dl-α-(hydroxymethyl)benzeneacetic acid, is a vital component of a number of pharmacologically important molecules. Atropine (dl-hyoscyamine), for example, is the 3-tropanol ester of racemic tropic acid. The pharmacologically active isomer, l-hyoscyamine, is the 3-tropanol ester of l-tropic acid. Because of the pharmacological difference between d- and l-hyoscyamine, there has been a great deal of

 0148-3919/84/0704-0731$3.50/0

interest in the development of an assay for the enantiomeric purity of atropine, which, in fact, is an assay to quantitate the d- and l-tropic acid moieties.

A number of researchers have reported the preparative resolution of tropic acid and atropine. Fodor and Csepreghy (1), for example, resolved tropic acid by the fractional crystallization of the diastereoisomeric D-(-)- and L-(+)-threo-1-(p-nitrophenyl)-2-amino-1,3-propanediol salts. Werner and Miltenberger (2) resolved tropic acid by using camphor-D-sulfonic acid. Although these approaches are successful on a preparative scale, they are not applicable to the analysis of pharmaceutical preparations or biological samples.

Landen and Caine (3) approached an analytical assay for atropine through the synthesis of diastereoisomeric urethane derivatives. They were able to form the diastereoisomers, but were unable to separate them via HPLC. To date, a survey of the literature shows no quantitative method available for the stereochemical determination of dl-tropic acid or dl-hyoscyamine.

The development and commercial introduction of HPLC chiral stationary phases (CSPs) such as the one described by Pirkle et al. (4), (R)-N-(3,5-dinitrobenzoyl)phenylglycine, offer a new approach to the solution of this problem. Pirkle et al. (5) and Wainer and Doyle (6) have shown that this CSP is capable of resolving the enantiomeric amides of α-methylarylacetic acids.

This paper reports the investigation of the applicability of this CSP to the resolution of some tropic acid amide and ester derivatives.

MATERIALS

Apparatus

The chromatography was performed with a Spectra-Physics (Santa Clara, CA, U.S.A.) Model 8000 liquid chromatograph equipped with an SP 8000 data system, a Spectra-Physics Model 8310 UV-visible detector set at 254 nm, and a temperature-controlled column compartment.

The column was a stainless steel, J.T. Baker-packed Pirkle covalent (R)-N-(3,5-dinitrobenzoyl)phenylglycine column (25 cm x 4.6 mm I.D.) with a silica packing of 5-μm spherical particles which were bonded through α-aminopropyl groups to the CSP.

Reagents

Atropine, dl-tropic acid, acetyl chloride, acetic anhydride, thionyl chloride, 1-naphthalenemethylamine (1-NAMA) and 1-naphthalenemethanol (1-NAMOL) were purchased from Aldrich (Milwaukee, WI, U.S.A.). dl-Tropicamide was a reference standard obtained from U.S. Pharmacopeial Convention, Inc. (Rockville, MD, U.S.A.). All HPLC organic solvents were purchased from Burdick & Jackson (Muskegon, MI, U.S.A.). The

remaining chemicals were reagent grade and were used as purchased.

METHODS (7)

Synthesis of Acetyltropic Acid (8)

Acetyl chloride (0.28 mole) was added at room temperature to 0.24 mole of tropic acid. The mixture was stirred until a clear liquid formed and then for an additional 5 min. The excess acetyl chloride was removed under a stream of nitrogen, and the resulting viscous oil was cooled until it produced a white crystalline solid.

Synthesis of Acetyltropyl Chloride (8)

Thionyl chloride (0.006 mole) was added to 0.005 mole of acetyltropic acid, and the mixture was stirred at 30°C until the evolution of gas ceased. The excess thionyl chloride was removed under vacuum, yielding a viscous oil which was used without purification.

The acid chloride was also synthesized by using oxalyl chloride (6). Oxalyl chloride (12.5 ml) was added to 0.001 mole of acetyltropic acid, and the mixture was heated at 60°C for 15 min. The excess oxalyl chloride was evaporated under a stream of nitrogen and the resulting viscous oil was used directly.

Synthesis of Acetyltropic Acid Naphthalenemethylamide

Acetyltropyl chloride was synthesized by starting with 0.001 mole of acetyltropic acid and following the oxalyl chloride procedure described above. Chloroform (15 ml) followed by 1-NAMA (0.006 mole) was added to the acid chloride and the solution was stirred overnight. The solution was then washed successively with two portions of 4N HCl and one portion of H_2O. The chloroform layer was collected and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded a colorless crystalline solid which was characterized by IR and NMR analysis.

Synthesis of Tropic Acid Naphthalenemethylamide

Acetyltropic acid naphthalenemethylamide synthesized above was refluxed on a steam bath for 1 h with 3N HCl. After the mixture was allowed to cool, the pH was adjusted to 9 with ammonium hydroxide and the mixture was extracted with chloroform. The chloroform layer was collected, dried over sodium sulfate and evaporated, yielding a colorless solid. The solid was recrystallized from ethyl acetate/hexane and characterized by using IR and NMR.

Synthesis of Acetyltropic Acid Naphthalenemethylester

Acetyltropyl chloride was synthesized by starting with 0.002 mole of acetyltropic acid and using the method involving thionyl

chloride described above. The resulting oil was dissolved in 25 ml of chloroform and 0.003 mole of l-NAMOL was added. The resulting solution was stirred overnight. The chloroform was washed successively with three portions of 4N HCl, with one portion of H_2O, and finally with a saturated sodium bicarbonate solution. The chloroform layer was collected and dried over sodium sulfate and the chloroform was evaporated. The resulting viscous oil was characterized by IR and NMR analysis.

Synthesis of Acetyltropicamide

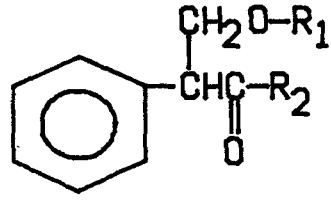
Acetic anhydride (0.008 mole) was added to 0.007 mole of tropicamide and the mixture was heated until clear. The resulting solution was mixed with chloroform and the chloroform layer was washed with 0.1N NaOH. The chloroform layer was collected, dried over sodium sulfate and evaporated to yield a colorless solid which was characterized by IR and NMR analysis.

Chromatographic Conditions

The compounds were chromatographed by using mobile phases of hexane and isopropanol mixed in various proportions (Table 1). The flow rate was 2 ml/min and the column temperature was 20°C.

TABLE 1

Chromatographic Results



Compound	R_1	R_2	k_1'[a]	α	R_s	Mobile Phase[b]
1 (amide)	acetyl	1-NAMA[c]	13.0	1.13	1.29	90:10
2 (amide)	H	1-NAMA	6.5	1.11	0.89	90:10
3 (ester)	acetyl	1-NAMOL[d]	5.3	1.00	0.00	95:5
4 (amide)	H	NENPMA[e]	15.3	1.03	0.39	95:5
5 (amide)	acetyl	NENPMA	12.9	1.08	0.57	95:5
6 (ester)	H	3-tropanol	33.6	1.00	0.00	85:15

[a]Capacity factor of first eluted enantiomer.
[b]The mobile phase was a mixture of hexane:isopropanol; the flow rate was 2 ml/min and the column temperature was 20°C.
[c]1-Naphthalenemethylamine.
[d]1-Naphthalenemethanol.
[e]N-Ethyl-N-(4-pyridinylmethyl)amine.

RESULTS

Attempts to synthesize the acid chloride of tropic acid directly were unsuccessful; it was necessary to first convert tropic acid to the O-acetyl derivative. Acetyl tropic acid was easily converted to the acid chloride by using either thionyl chloride or oxalyl chloride; the desired amide or ester

derivative was then readily obtained by the addition of the appropriate amine or alcohol.

The enantiomeric amides formed from dl-acetyltropyl chloride and l-NAMA, compound 1, Table 1, were resolved by the CSP; the separation factor (α) = 1.13. To determine the effect of the O-acetyl function on this separation, the acetyl moiety was removed by acid hydrolysis (8) and the resulting product, compound 2, was chromatographed. There was essentially no change in the resolution of the two compounds; α = 1.13 vs 1.11 for 1 and 2, respectively. However, there was a decrease in the resolution factor (R_s) when the acetyl group was removed, i.e., 1.29 vs 0.89 for 1 and 2, respectively.

The enantiomeric esters formed from dl-acetyl tropic acid chloride and l-NAMOL, compound 3, were not resolved by the CSP under chromatographic conditions similar to those which resolved the amides. Atropine, 6, the tropine ester of dl-tropic acid, was also not resolved by the CSP.

The anticholinergic agent tropicamide, compound 4, which is used in ophthalmic preparations, was resolved directly on the CSP without derivatization; α = 1.03. This compound is the N-ethyl-N-(4-pyridinylmethyl)amide of dl-tropic acid. To determine the effect of an O-acetyl group on this resolution, tropicamide was derivatized by using acetic anhydride. There was a slight increase in the resolution of the resulting compound, 5; α = 1.08 vs 1.03, for 5 and 4, respectively; there was also an increase in R_s, 0.57 vs 0.39.

DISCUSSION

Pirkle et al. (5,9) have suggested that the chiral recognition mechanism for amides on this CSP involves the formation of a CSP-solute complex which is dependent upon a dipole-dipole interaction between the 3,5-dinitrobenzoylamide moiety on the CSP and the amide moiety on the solute. The steric environment at the chiral center determines the stability of the complex, and, thus, the resulting resolution and order of enantiomeric elution.

Work in this laboratory on the resolution of α-methylaryl-acetic acids (6) supports this postulate. It was found that the amide derivatives of the compounds studied were resolved on the CSP, whereas corresponding ester derivatives were not. This difference was explained on the basis of the difference in dipole moments between amides and esters and the resulting difference in the strength of the interaction between the CSP and the solute, which, in turn, affects the ability of the CSP to differentiate between the enantiomers.

The results of this study are consistent with the proposed chiral recognition mechanism. The slight difference in resolution between the amides of tropic acid, 2 and 4, and the amides of O-acetyltropic acid, 1 and 5, seems to indicate that neither hydrogen bonding involving the hydroxyl hydrogen in the unacetylated molecule nor hydrogen bonding involving the carbonyl function in the acetylated derivative plays a key role

in the formation of the CSP-solute complex. However, since these groups are part of the steric environment surrounding the chiral center, they probably play a role in the chiral recognition process once the complex is formed.

On the other hand, the fact that the amides are resolved and the esters, 3 and 6, are not, suggests that the dipole strength of the carbonyl derivative of tropic acid is a major factor in the formation of the CSP-solute complex. The amide-amide interaction appears to lead to the formation of a strong CSP-solute complex which promotes the chiral recognition process. The amide-ester dipole interaction, however, produces a weaker complex; compare, for example, the capacity factors of amide 1 and ester 3. Therefore, there is no effective discrimination between the ester enantiomers.

In light of the proposed chiral recognition mechanism, it is not surprising that atropine was not resolved on this CSP. The solution to this analytical problem perhaps awaits the development of alternative CSPs that are effective in the resolution of esters as a class.

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