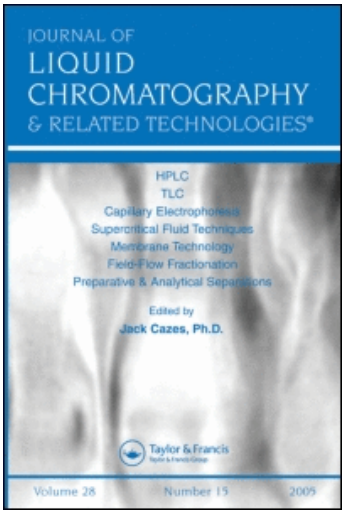


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Reversed-Phase Liquid Chromatographic Behavior Of The New 5-HT_1D Receptor Agonist Rizatriptan Benzoate and Its potential Process Impurities

V. Antonucci[a]; L. Wright[a]; P. Toma[a]
[a] Analytical Research Department, Merck Research Laboratories Merck & Co., Inc., Rahway, NJ

To cite this Article Antonucci, V. , Wright, L. and Toma, P.(1998) 'The Reversed-Phase Liquid Chromatographic Behavior Of The New 5-HT_1D Receptor Agonist Rizatriptan Benzoate and Its potential Process Impurities', Journal of Liquid Chromatography & Related Technologies, 21: 11, 1649 — 1670
To link to this Article: DOI: 10.1080/10826079808001250
URL: http://dx.doi.org/10.1080/10826079808001250

J. LIQ. CHROM. & REL. TECHNOL., 21(11), 1649–1670 (1998)

THE REVERSED-PHASE LIQUID CHROMATOGRAPHIC BEHAVIOR OF THE NEW 5-HT_1D RECEPTOR AGONIST RIZATRIPTAN BENZOATE AND ITS POTENTIAL PROCESS IMPURITIES

Vincent Antonucci, Lisa Wright, Pascal Toma

Analytical Research Department
Merck Research Laboratories
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0914

ABSTRACT

The reversed-phase chromatographic behavior of the powerful new anti-migraine drug rizatriptan benzoate and its potential impurities has been studied. Molecular dynamics calculations were used to explain the elution order of the two regioisomers of rizatriptan formed during its synthesis in terms of conformational differences. Further, van't Hoff plots for a mixture of the two regiosiomers and one potential process impurity were non-linear (R = 0.937 - 0.965) when chromatographed on an SB-Phenyl column. However, van't Hoff plots for the same analytes were linear ($R \geq 0.996$) when chromatographed on an C_8 column. The break in the van't Hoff plots generated with an SB-Phenyl phase occurs at ambient temperature (~25°C) and is attributed to changes in stationary phase morphology as a function of temperature.

The SB-Phenyl phase is believed to orient itself in a much more rigid state at sub-ambient temperatures than at temperatures above ambient, which results in the observed reduction in the enthalpy of interaction for analytes at sub-ambient temperature. A corresponding decrease in separation factor (ln α) between rizatriptan regioisomers with increasing temperature is observed as the shape selectivity of the SB-phenyl stationary phase decreases.

INTRODUCTION

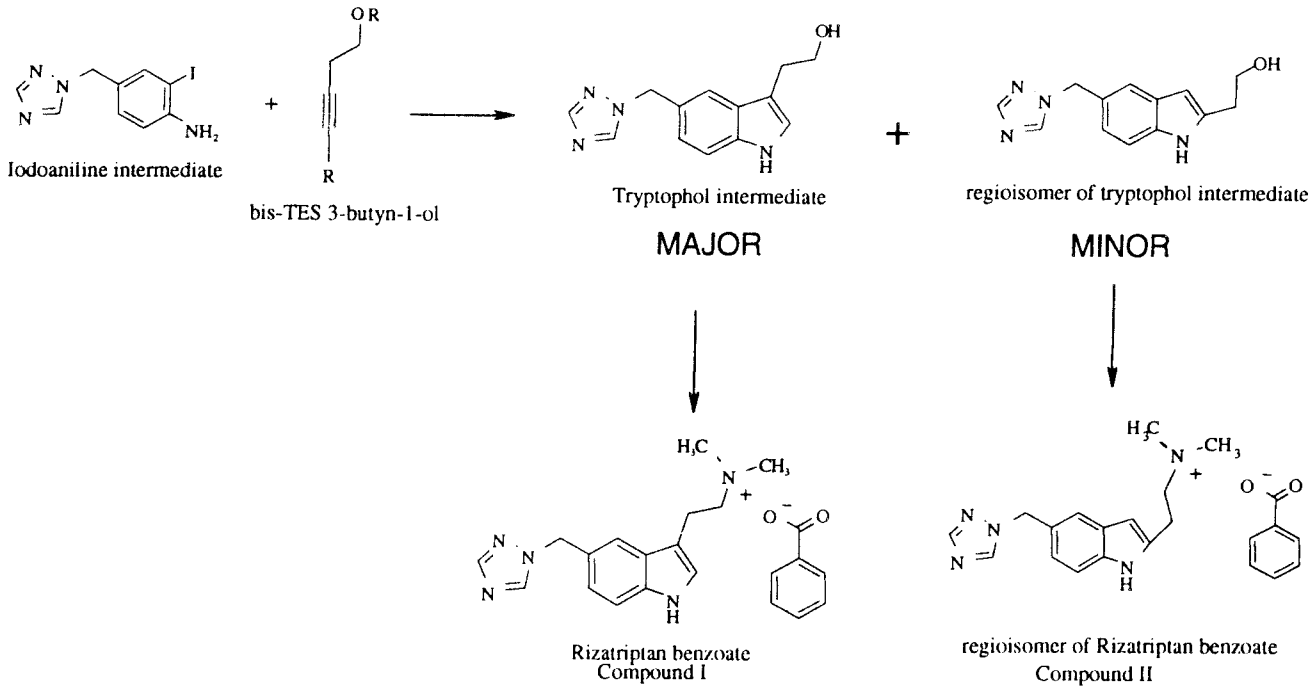
Over the past decade, recent advances in reversed-phase liquid chromatography have dramatically increased its applications.[1] Accordingly, considerable effort has been made to explain the mechanisms responsible for retention in reversed-phase separations, which in turn has resulted in significant debate within the chromatographic literature. Solvophobic theory describes the equilibrium constant for reversed-phase retention as a two-step transfer mechanism in which solutes migrate into and out of solute-sized cavities in the mobile phase.[2] Solute adsorption with the stationary phase is not a major consideration of the theory. Subsequently, an alternate theory termed the "partitioning model" was developed to include the effects of stationary phase structure on retention.[3,4] The theory uses statistical thermodynamics to consider the three-dimensional stationary phase chain organization as a function of mobile phase composition. Solute transfer from the mobile phase into stationary phase cavities is controlled by geometrical constraints offered by the solutes and stationary phase.

The effect of temperature on the stationary phase chain orientation and retention has been reviewed.[5] Specifically, the slot model developed by Sander and Wise[6] demonstrates that selectivity for polyaromatic hydrocarbons on linear alkyl-substituted bonded-phases is greatly effected by temperature. The rigidity of alkyl bonded phases increases inversely with temperature. As a result, the selectivity for planar and linear solutes increases as a function of stationary phase rigidity. Conversely, bulkier solutes are prohibited from entering into the stationary phase and the resulting enthalpic interactions between solute and stationary phase (thus retention) are minimized. Other reports of low temperature-related effects on stationary phase morphology and selectivity on linear alkyl-substituted reversed-phase columns have been recently published.[7-11]

The siloxane bond which connects the bonded phase to the support is easily hydrolyzed by low pH (2-3) mobile phases.[12] However, some applications of reversed-phase liquid chromatography with ionizable solutes require such a pH range to minimize secondary equilibria (ionic interactions) with the exposed silanols of the silica support. The recent emergence of highly end-capped bonded phases has minimized the potential acid-hydrolysis of the stationary phase, prolonging the lifespan of columns.[13] Further, C_{18} phases which have been sterically-protected with diisopropyl or diisobutyl functional groups demonstrate even greater stability to low pH and high temperature than do conventional monomeric dimethyl end-capped C_{18} phases.[14] The use of ion-pair reagents at acidic pH has further extended the applications of bonded-phase liquid chromatography to include highly polar and strongly basic analytes which were previously analyzed by ion exchange chromatography.[15] A counter ion of opposite charge to the analyte is added as a modifier to the mobile phase to form a neutral species which is more soluble in the organic component of the mobile phase, thus increasing retention. The capacity factor (k') of the solute is controlled by the concentration of the counter-ion, pH, and the percent concentration of organic modifier present in the mobile phase.

The pharmaceutical industry generates many ionizable drug substances which are ideal candidates for ion-pair reversed-phase chromatography. Many of these Compounds must be isolated at an extremely high purity from complicated in-process matrices, which often requires sensitive chromatographic methods to resolve structurally-similar impurities. Therefore, an understanding and experimental control of chromatographic selectivity is essential to achieving these required separations. This paper deals with the separation of some of the in-process intermediates and impurities potentially present in rizatriptan benzoate [5-[1(1,2,4-triazolyl)methyl]-3-(N,N-dimethylaminoethyl)indole benzoate], whose structure and synthesis are shown in Scheme 1.[16,17] Rizatriptan benzoate is a new potent and selective 5-HT$_1$D receptor agonist with good oral bioavailability and rapid onset of action for the treatment of migraine.[18]

The coupling of *iodoaniline intermediate* and *bis-TES 3-butyn-1-ol intermediate* (Scheme I) in the synthesis of rizatriptan benzoate generates the *tryptophol intermediate* and a small percentage of the regioisomer of the *tryptophol intermediate* (substitution at the 2-position of the indole ring).[17] This regioisomer is subsequently converted into the regioisomer of rizatriptan benzoate (termed **Compound II**) during downstream processing and then controlled by the testing procedures for rizatriptan benzoate (termed **Compound I**). Thus it is critical to develop a sensitive means of quantitating trace amounts of **Compound II** in bulk **Compound I**.



Scheme 1. Chemical transformations occurring during the synthesis of Rizatriptan benzoate which result in the formation of Compounds I and II.

A literature survey revealed a few recent reports of investigations into the reversed-phase separation of similar achiral regioisomers. The selectivity of five regioisomers of phenethylamine was found to be controlled by the length of the carbon chain attached to the aromatic ring.[19] The ability to separate two benzodiazepiane regioisomers, which differ only in methyl group position, was attributed to the magnitude of dielectric interactions between the solutes and the mobile phase.[20] It was therefore concluded that the separation factor (α) could not be improved for benzodiazepianes by choosing an alternate stationary phase and/or an alternate mobile phase.

Molecular modeling has been used previously to assist in the explanation of both the elution order and mechanism of chromatographic separations.[21-23] The magnitude of the interaction energy calculated between α-CD, β-CD, and γ-CD (cyclodextrins) and a C_{18} bonded phase, as well as the degree of hydration of the cyclodextrins, were found to directly correlate with retention order.[21] Molecular modeling and ^{1}H NMR were used to explain the enantiomeric separation of propranolol by SFC and LC. It was determined after modeling both the stationary phase, analytes, and the interaction between analytes and stationary phase that (R)-propranolol has more sites of interaction with the (S)-CSP stationary phase than (S)-propranolol, hence it should be retained longer.[22]

The retention characteristics of a series of phenylpropyl and methoxyphenylpropyl bonded phases under normal phase elution conditions for C_{60} and C_{70} fullerenes was predicted from calculated low-energy conformations and associative energies, and was found to agree with experimental results.[23] Retention on the various phenyl phases was found to be directly related to the contact surface area between the analyte and stationary phase, which is a function of the conformational similarities of the analyte and stationary phase. This paper will use both conformational analysis and chromatographic experimentation to elucidate the retention behavior of rizatriptan benzoate and two potential process intermediates.

EXPERIMENTAL

Materials

Rizatriptan benzoate and its related intermediates have been prepared at various departments within Merck Research Laboratories.[24] All solvents used for chromatography were of HPLC grade (Fisher Scientific, Fairlawn, NJ). Reagent grade trifluoroacetic acid (Fisher Scientific), phosphoric acid, 85% (J.T. Baker, Phillipsburg, NJ), and hexanesulfonic acid (PIC® B-6 Low UV reagent, Waters

Corporation, Milford, MA) were used as mobile phase additives. Zorbax SB-phenyl columns (25 cm x 4.6 mm i.d., 5 mm packing) were obtained from MAC-MOD Analytical (Chadds Ford, PA) and Symmetry C8 columns (15 cm x 3.9 mm i.d., 5 mm packing) were obtained from Waters.

Instrumentation

Two different HPLC systems were used during method development and validation. One HPLC system consisted of Shimadzu components including an SIL-10A autoinjector, SCL-10A system controller, LC-10AS gradient pumps, and SPD-10AV UV/VIS detector. The second HPLC system included a Thermoseparations AS3000 autosampler and P4000 gradient pump, with a Spectraflow 757 UV/VIS detector (Kratos Analytical). A model 7955 HPLC column heater/chiller (Jones Chromatography, Lakewood, CO) was used for the temperature studies. Flow rate was kept constant at 1.5 mL/min. Samples were dissolved in 90% water/ 10% acetonitrile (v/v) diluent at 1 mg/mL concentration. Ultraviolet detection at 280 nm was used for all systems.

Molecular Modeling Studies

All of the computations performed in this study were determined with Cerius$^{2™}$ Release 2.0 developed by BIOSYM/Molecular Simulations on various Silicon Graphics computers available at Merck & Co., Inc.[25] Energy minimizations and molecular dynamics were calculated using the Universal Force Field and charges were assigned with the Charge Equilibration Method.[26,27] Optimized conformations (lowest energy) were determined through dynamic simulations followed by energy minimization and charge re-calculation. This process was repeated at least ten times for each molecule to ensure that the lowest energy conformation possible was found.

Chromatographic Methods

Four chromatographic systems (Systems A - D) were developed to study the retention behavior of a mixture of four analytes. Isocratic elution conditions for all four methods were adjusted such that **Compound I** retention times were maintained between 5 - 7 minutes. The mixture used for measurements made with Systems A and B was approximately 1 mg/mL of the free base of **Compound I**, 0.1 mg/mL of the benzoate of **Compound II**, 0.6 mg/mL of tryptophol intermediate, and benzoic acid from **Compound II**. The mixture used for measurements made with Systems C and D was approximately 1 mg/mL of the free base of **Compound I**, 0.3 mg/mL of

Table 1

Chromatographic Systems Developed to Investigate the Retention Behavior of Compound I, Compound II, and *Tryptophol Intermediate*

System A: *Mobile phase* - 84% (0.1% trifluoroacetic acid (v/v) in water) / 16% acetonitrile (v/v)
Column - SB-Phenyl

System B: *Mobile phase* - 88% (0.1% phosphoric acid (v/v) in water) / 12% acetonitrile (v/v)
Column - SB-Phenyl

System C: *Mobile phase* - 92% (0.1% trifluoroacetic acid (v/v) in water) / 8% acetonitrile (v/v)
Column - C_8

System D: *Mobile phase* - 87.5% (0.1% phosphoric acid (v/v) in water, 10 mM hexanesulfonic acid) /
12.5% acetonitrile (v/v); *Column* - C_8

the benzoate of **Compound II**, 0.2 mg/mL of tryptophol intermediate, and benzoic acid from **Compound II**. These systems are described in Table 1. The methods studied used either an SB-phenyl phase or a C_8 phase to determine whether the choice of reversed-phase offers selectivity advantages for the analytes. A minimum of two repeat determinations of retention time were made at each temperature. The effect of three different ion-pairing reagents on retention for the two stationary phases was also evaluated.

RESULTS AND DISCUSSION

Role of Molecular Conformations

In chromatographic systems A-D, **Compound I** is observed to consistently elute prior to **Compound II** (Figure 1). Molecular modeling was used to compare the conformations of these molecules in hopes of relating the differences observed to reversed-phase selectivity. The lowest energy conformation of both **Compound I** and **Compound II** was determined and is represented in Figure 2. The molecular energy calculated for **Compound I** was 90 kcal/mole, while for **Compound II** it was 60 kcal/mole. Qualitatively, the

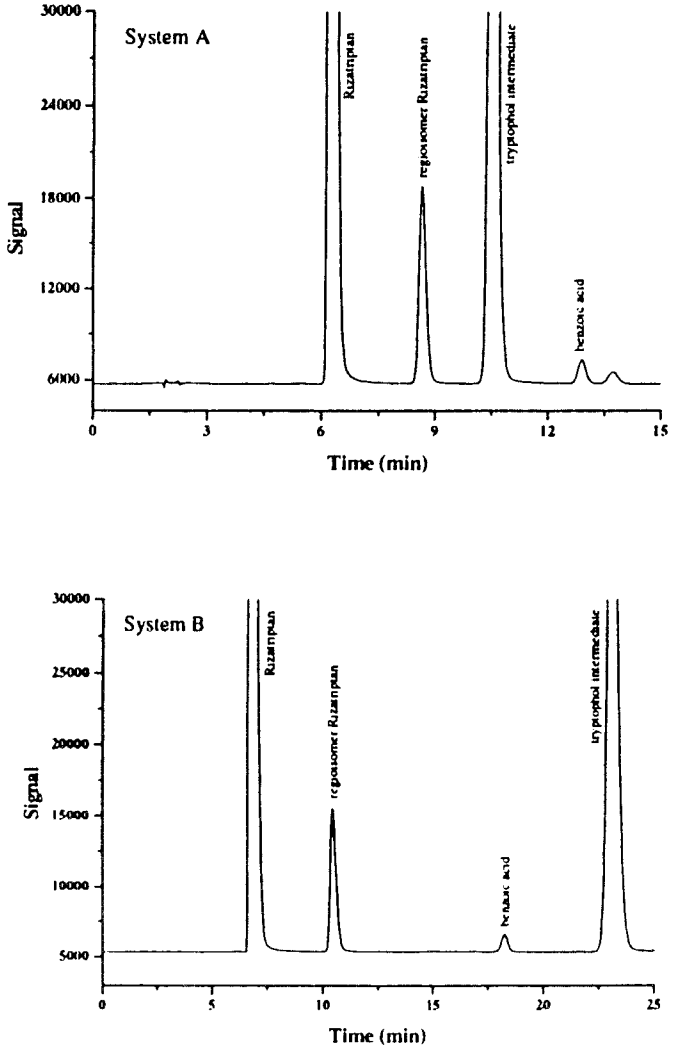


Figure 1. Typical chromatograms obtained at 25°C for Systems A - D.

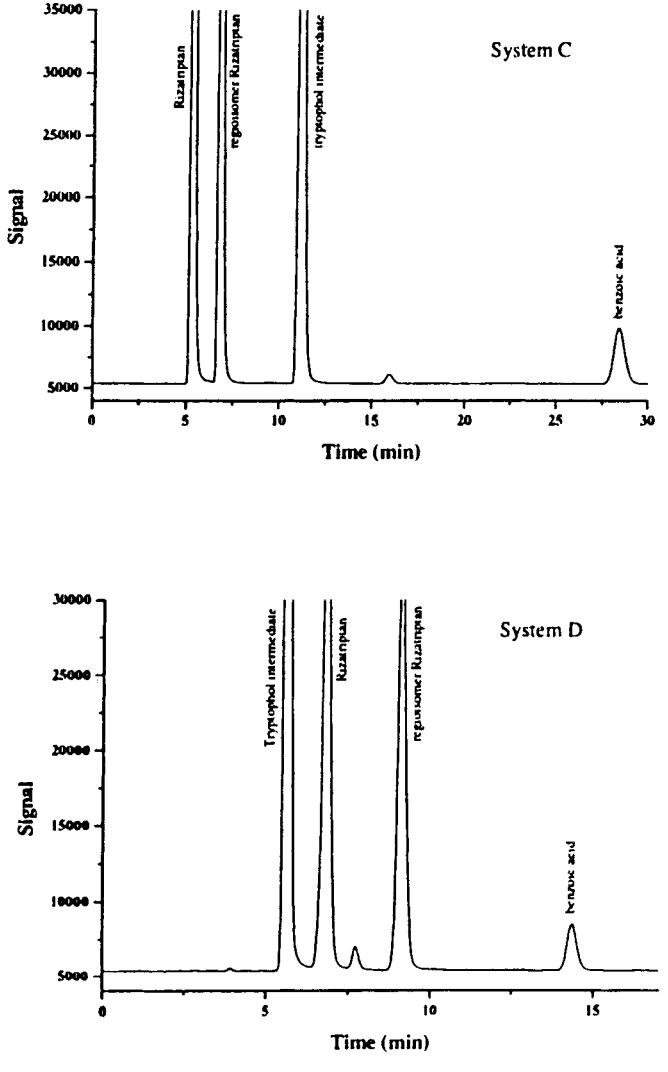
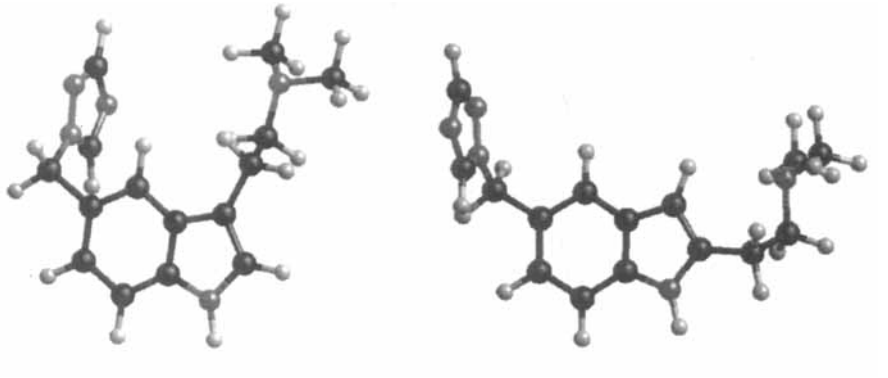


Figure 1 (cont.). Typical chromatograms obtained at 25°C for Systems A - D.



Compound I **Compound II**

Figure 2. Lowest energy conformations calculated for **Compounds I** and **II**.

energy-minimized structure of **Compound II** is more planar than that of **Compound I** which should allow for a greater surface area of the molecule to be in contact with the stationary phase at any given time. It is therefore postulated that the elution order observed for the regioisomers is governed by the average available contact area between the analyte and the stationary phase, which is controlled by conformational differences between molecules.

van't Hoff Plots

The relationship between solute capacity factor and partial molar free energy during solute transfer between stationary phase and mobile phase is well known as:

$$\ln k' = - (\Delta G°/RT) + \ln \Phi$$

where Φ is the phase ratio (volume of stationary phase / volume mobile phase).[28] This relationship also may be represented by the van't Hoff equation:

$$\ln k' = - (\Delta H°/RT) + \Delta S°/R + \ln \Phi$$

where k' is the analyte capacity factor, R is the gas constant (expressed in cal/(°K mol)), and $\Delta H°$ and $\Delta S°$ are the enthalpy and entropy of analyte transfer from the mobile phase to the stationary phase, respectively. A van't

Hoff plot of ln k' vs. 1/ T(°K) will generally be linear with slope - (ΔH°/R) and intercept (ΔS°/R + ln Φ) due to a single retention mechanism governing the separation. Numerous reports of linear van't Hoff plots have been published for monomeric C_{18} phases, but ΔS° is often not reported due to ambiguity in calculating the phase ratio of commercial phases.[29-32]

Chromatographic Behavior on an SB-Phenyl Phase

The effect of ion pairing reagent upon retention of the four component mixture on an SB-phenyl phase was evaluated with System A and System B. Trifluoracetic acid (TFA) is more chaotropic (less hydration due to high polarizablity) than phosphoric acid. Therefore, mobile phases containing TFA should disrupt the hydration shell of protonated analytes better than phosphoric acid-containing mobile phases, which results in greater enthalpic interactions of the analyte with the stationary phase.[33,34] For this reason, more acetonitrile is necessary in System A than in System B (16 vs. 12% (v/v)) to retain **Compound I** with a similar capacity factor.

Figures 3a and 3b are van't Hoff plots for the data over the temperature range of 0 to 50°C ($0.00366 \geq 1/T \geq 0.00310$). Individual data points were plotted at each temperature in all van't Hoff plots to provide visual evidence of the high reproducibility of measurements. Benzoic acid retention data from the systems under consideration is plotted in all van't Hoff plots as a small molecule probe and will be discussed later in this paper. Both data sets indicate a deviation from linearity (R = 0.937 - 0.965), with a break point at approximately 25°C ($1/T \sim 0.00336$). Deviations from linearity in van't Hoff plots have been reported for temperature studies of reversed-phase stationary phases.[10,35-39] The curved plots have been attributed to reversible, morphological changes in the stationary phase as a function of temperature, stationary phase bonding density, and solvent release processes.[40] Dorsey cautions, however, that such morphological changes in the bonded phase as a function of temperature do not necessarily predict a change in the intrinsic retention mechanism, but may be due to factors such as a change in phase ratio.[41]

A linear relationship was assumed for the data in Figures 3a and 3b in order to facilitate calculation of approximate enthalpic and entropic terms, which are presented in Table 2. In both Systems A and B, the enthalpic term for both **Compound I** and the tryptophol intermediate is equivalent, while the enthalpic term for **Compound II** is over 300 cal/(°K mol) larger.

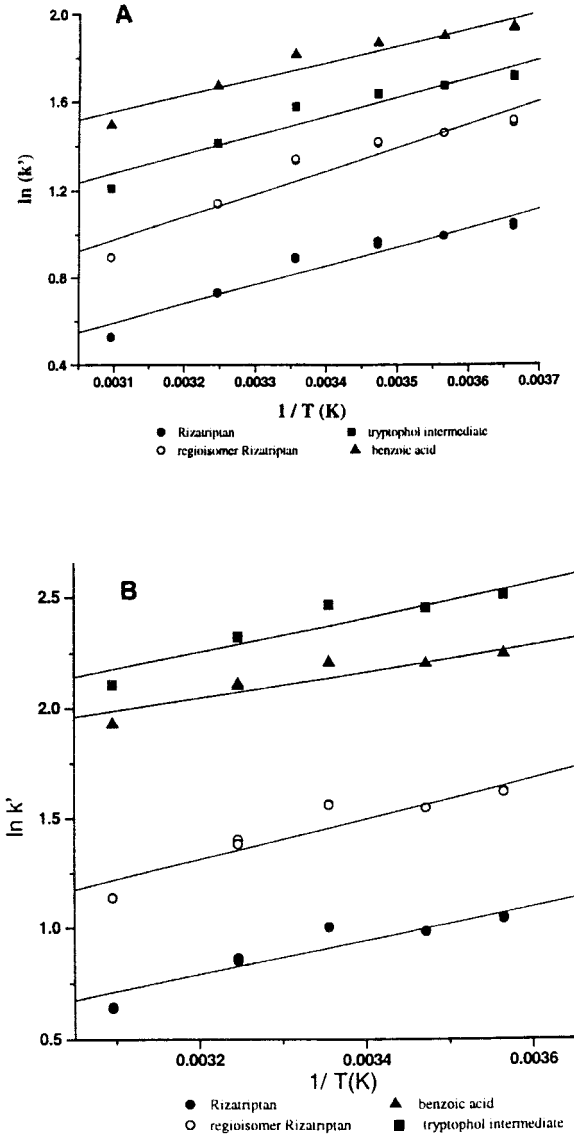


Figure 3. A. van't Hoff plot of ln k' vs 1/ T (°K) for the data obtained with System A, assuming a linear relationship. B. van't Hoff plot of ln k' vs 1/ T (°K) for the data obtained with System B, assuming a linear relationship.

Table 2

Enthalpy and the Entropic Term for Compound I, Compound II, and *Tryptophol Intermediate* in (cal/°K mol) on SB-Phenyl Stationary Phase

Analyte	System A		System B	
	$\Delta H°$	$\Delta S° + \ln\phi$	$\Delta H°$	$\Delta S° + \ln\phi$
Compound **I**	- 1743.6	- 4.2	- 1517.8	- 3.3
Compound II	- 2082.0	- 4.5	- 1836.2	- 3.3
Tryptophol intermediate	- 1707.6	- 2.8	- 1506.5	- 0.3

Both **Compound I** and the *tryptophol intermediate* are substituted in the 3-position of the indole ring and presumably have similar conformations and similar enthalpy, while **Compound II** is a more planar structure which allows for greater surface area of contact with the stationary phase and greater enthalpy. However, **Compounds I** and **II** are protonatable at pH 2, and are therefore retained less than the neutral *tryptophol intermediate*.

These results confirm the predicted elution order of the rizatriptan regioisomers from the molecular modeling studies. No significant selectivity advantages were observed for System B over System A while enthalpic interactions were reduced, so phosphoric acid-containing mobile phases will no longer be considered.

If the data in Figure 3a is divided into the two linear regions (each with $R > 0.990$) present on either side of 25°C ($1/T \sim 0.00336$) as seen in Figure 4, greatly different enthalpic and entropic terms are calculated for each region (Table 3). While the energies calculated in Table 3 are approximations from small data sets, the general observation may be made that the enthalpy in the $\geq$ 25°C ($1/T \leq 0.00336$) region is significantly greater than it is in the sub-ambient region.

It is suggested that at sub-ambient temperatures the stationary phase is more extended and rigid due to decreased molecular motions, which lead to increased aromatic interactions between neighboring phenyl groups.[42,43] As a result, analyte penetration within the stationary phase is hindered due to steric effects and subsequent enthalpic interactions are reduced. At temperatures above ambient, the kinetic activity of the stationary phase phenyl groups

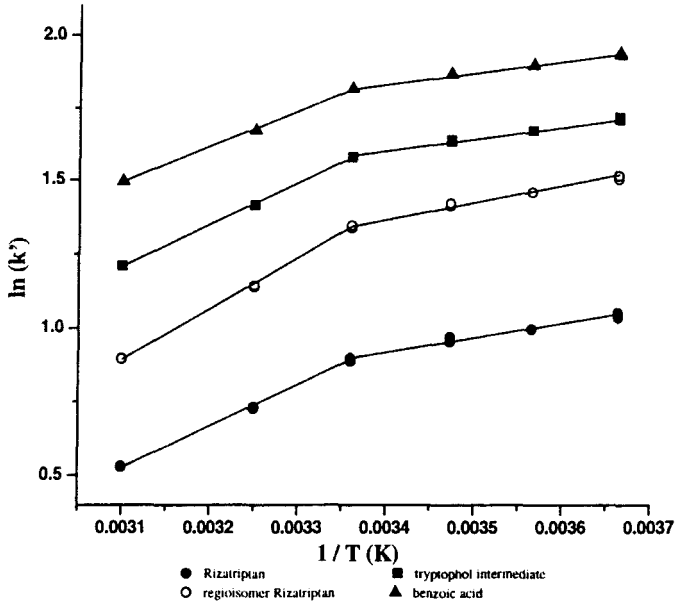


Figure 4. van't Hoff plots of ln k' vs 1/ T (°K) for the data obtained with System A above and below 25°C, as indicated. ΔH° and ΔS° + ln Φ were calculated for each region.

Table 3

Approximations of Enthalpy and the Entropic Term for Compound I, Compound II and the *Tryptophol Intermediate* in (cal/°K mol) on an SB-Phenyl Stationary Phase*

Analyte	Below 25°C		Above 25°C	
	ΔH°	ΔS° +ln ϕ	ΔH°	ΔS° +ln ϕ
Compound I	- 954.8	- 1.4	- 2782.5	- 7.6
Compound II	- 1101.6	- 1.0	- 3404.5	- 8.8
Tryptophol intermediate	- 888.4	0.2	- 2821.1	- 6.3

* At temperatures above and below 25°C using chromatographic system A.

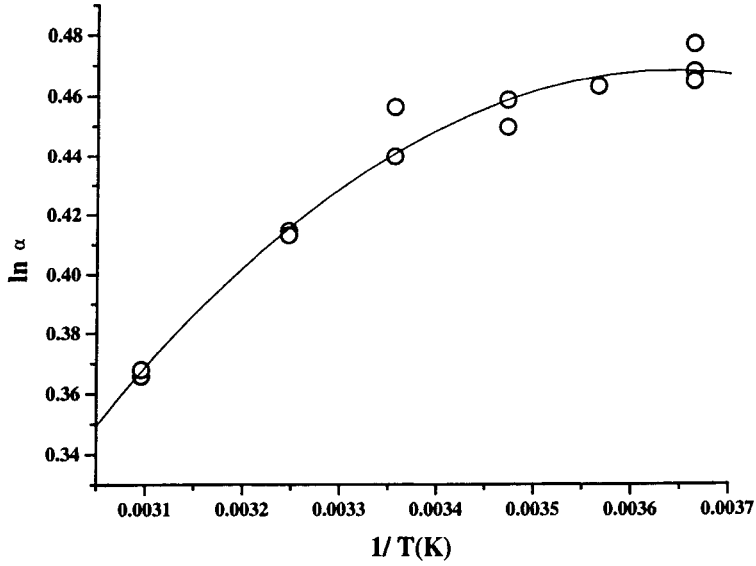


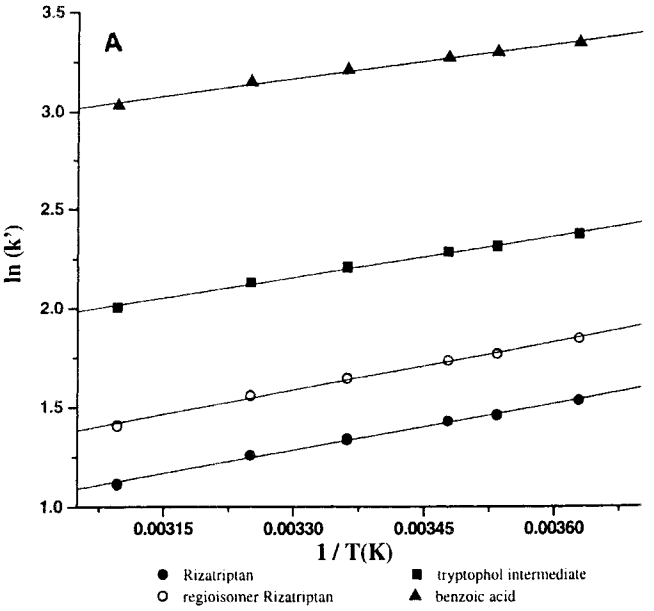
Figure 5. Plot of ln α vs. 1/ T (°K) for the separation of **Compound I** and **Compound II** using System A.

increases, allowing for greater spacing of the bonded phase chains. This allows increased analyte penetration within the stationary phase and thus greater enthalpic interactions due to increased surface area of contact between the analyte and the stationary phase.

The postulated dependence of stationary phase rigidity on temperature was investigated *via* a plot of the ln a (separation factor) vs. 1/T (°K) for **Compounds I and II** with System A (Figure 5). Chromatographic selectivity is observed to monotonically decrease with increasing temperature. Closer inspection of the data at temperatures below 25°C ($1/T \geq 0.00336$), indicates a higher and more constant separation factor than for temperatures above 25°C ($1/T \leq 0.00336$), where the separation factor decreases much more rapidly.

Chromatographic Behavior on a C_8 Phase

These observations are consistent with the hypothesis that stationary phase rigidity, and ultimately analyte shape selectivity, are functions of temperature on the SB-phenyl phase.



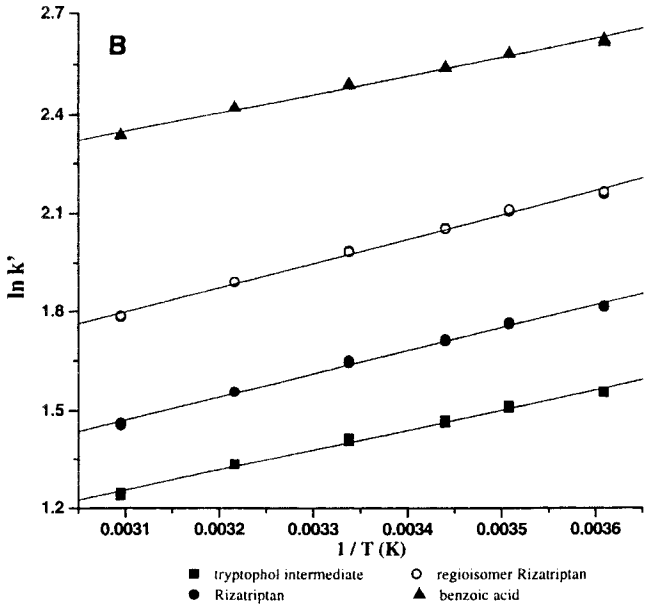


Table 4

Enthalpy and the Entropic Term for Compound I, Compound II and *Tryptophol Intermediate* in (cal/°K mol) on a C_8 Stationary Phase

Analyte	System A		System B	
	ΔH°	ΔS° + Inϕ	ΔH°	ΔS° + Inϕ
Compound I	- 1557.0	- 2.6	- 1381.9	- 1.4
Compound II	- 1620.1	- 2.2	- 1471.0	- 1.0
Tryptophol intermediate	- 1387.5	- 0.3	- 1214.9	- 1.3

The same four component mixture was chromatographed on a C_8 column under System C and System D conditions and van't Hoff plots constructed (Figures 6a and 6b). The plots are highly linear (R = 0.996 - 0.998), unlike the data collected with the SB-phenyl phase (Figures 3a, 3b, and 4). Further, it is worth noting that all benzoic acid van't Hoff plots are quite similar to those of the rizatriptan-related species on both the SB-phenyl and C_8 phases (Figures 3a, 3b, 4, 6a, and 6b). These data suggest that thermal characteristics unique to the SB-phenyl stationary phase are responsible for the observed non-linearity in van't Hoff plots for all analytes considered, not any analyte-specific characteristics such as conformation or solvation state.

The magnitude of the enthalpic interaction is smaller on the C_8 phase than for the SB-phenyl phase as determined by the amount of acetonitrile needed for elution in System A (16%) as compared to System C (8%). The values of ΔH° and ΔS° + ln Φ for System C are in Table 4 and for System A are in Table 2.

The entropic terms are more positive than those calculated for the SB-phenyl phase because the phase ratio (Φ) is ca. 2-3 fold greater for the C_8 phase due to increased carbon loading (12% carbon for Symmetry C8 vs. 5.5% carbon for SB-Phenyl).[13,44-45] The difference in enthalpic interactions for **Compound II** and **Compound I** is only 60 - 90 cal/(°K mol) on the C_8 phase.

Figure 6 (left). A. van't Hoff plot of ln k' vs 1/ T (°K) for the data obtained with System C. B. van't Hoff plot of ln k' vs 1/ T (°K) for the data obtained with System D.

It is also noted that the enthalpy value for **Compound I** and the *tryptophol intermediate* are more dissimilar than calculated for the SB-Phenyl phase. These results suggest that the C_8 phase displays less shape selectivity for the various conformational differences of the rizatriptan-related species than observed for the SB-phenyl phase and is thus a poorer choice of stationary phase for this separation.

As a final experiment, a larger surfactant-like ion-pairing reagent was evaluated on the C_8 phase (System D) to determine the effect on retention. As anticipated, ion-pairing of the rizatriptan-related species with hexanesulfonic acid does increase the amount of acetonitrile required for elution with a similar k' (12.5 % (v/v) in System D vs. 8% (v/v) in System C). While the elution order for the ionizable species is constant for Systems A-D (**Compound I**, **Compound II**, benzoic acid), the elution order of the *tryptophol intermediate* (non-ionizable) varies with the system considered (Figure 1). It is eluted last of the four analytes when phosphoric acid modifier is used (System B), third when TFA is used on both stationary phases considered (Systems A and C), and first when hexanesulfonic acid is used (System D). The data from Systems A and C demonstrate that the observation is independent of choice of stationary phase and rather must be related to the ion-pairing reagent employed.

It is postulated that retention characteristics observed for the *tryptophol intermediate* are related to the ability of the hydrophobic portion of the ion-pairing reagent to coat the stationary phase, creating an additional retention mechanism of ion-exchange at low pH. On the basis of chaotropicities, hexanesulfonic acid is best capable of hydrophobically-interacting with the stationary phase, followed by TFA then phosphoric acid. Since the neutral *tryptophol intermediate* cannot benefit from the additional ion-exchange mechanism as the rizatriptan species do, it elutes first when hexane sulfonic acid modifier is used in System D. The ion-exchange effect is much smaller with TFA modifier, as seen for Systems A and C, hence the neutrality of the tryptophol intermediate reduces retention by much smaller amounts. The effects of tryptophol neutrality are negligible with phosphoric acid modifier (System D) because the modifier does not appreciably coat the stationary phase surface.

CONCLUSIONS

Rizatriptan benzoate is a new potent and selective 5-HT_1D receptor agonist for the treatment of migraine. The synthetic pathway to Rizatriptan benzoate generates positional isomers (**Compound I** and **Compound II**) which have been chromatographically resolved on the basis of the conformational

differences of the molecules. The elution order of the regioisomers can be predicted by a molecular model representing the conformational differences between regioisomers. The basis of the separation is the magnitude of van der Waals interaction energy of the analytes with the stationary phase, which is controlled by molecular conformation. These conformational differences result in a 320 - 340 cal/ (°K mol) increase in enthalpic interactions for **Compound II** as compared to **Compound I** and tryptophol intermediate on an SB-Phenyl stationary phase and 60 - 90 cal/ (°K mol) increase in enthalpic interactions on a C_8 phase. van't Hoff plots on SB-Phenyl columns are non-linear (R = 0.937 - 0.965), but are linear on a C_8 phase (R = 0.996 - 0.998) for **Compound I**, **Compound II**, tryptophol intermediate, and benzoic acid regardless of mobile phase additives. The shape selectivity of the SB-phenyl stationary phase for rizatriptan regioisomers was observed to decrease with increasing temperature. It is concluded that the thermal properties of the stationary phases considered are responsible for the observed thermodynamic behavior of analytes in the chromatographic systems considered. However, within a given chromatographic system, the selectivity between regioisomers is driven by analyte conformational differences.

ACKNOWLEDGMENTS

The authors would like to acknowledge Richard Egan, Nelu Grinberg, and Richard Thompson for many helpful discussions during the completion of this work.

REFERENCES

1. R. E. Majors, LC-GC, **6**, 298-302 (1988).

2. C. Horvath, W. Melander, I. Molnar, J. Chromatogr., **125**, 129-156 (1976).

3. D. E. Martire, R. E. Boehm, J. Phys. Chem., **87**, 1045-1062 (1983).

4. K. A. Dill, J. Phys. Chem., **91**, 1980-1988 (1987).

5. B. Ooms, LC-GC, **14(4)**, 306-324 (1996).

6. L. C. Sander, S. A. Wise, Anal. Chem., **61**, 1749-1754 (1989).

7. W. S. Hancock, R. C. Chloupek, J. J. Kirkland, L. R. Snyder, J. Chromatogr. A, **686**, 31-43 (1994).

8. W. S. Hancock, R. C. Chloupek, J. J. Kirkland, L. R. Snyder, J. Chromatogr. A, **686**, 45-59 (1994).

9. L. C. Sander, N. E. Craft, Anal.Chem, **62**, 1545-1547 (1990).

10. K. B. Sentell, A. N. Hendersen, Anal. Chim. Acta, **246**, 136-149 (1991).

11. H. Ohta, Y. Saito, K. Jinno, H. Nagashima, K. Ito, Chromatographia, **39(7/8)**, 453-459 (1994).

12. J. L. Glajch, J. J. Kirkland, J. Kohler, J. Chromatogr., **384**, 81-90 (1987).

13. J. J. Kirkland, J. L. Glajch, R. D. Farlee, Anal. Chem., **61**, 2-11 (1988).

14. J. J. Kirkland, J. W. Henderson, J. Chromatogr. Sci., **32**, 473-480 (1994).

15. D. A. Skoog, **Principles of Instrumental Analysis**, 3rd ed., p. 813 (1985).

16. L. J. Street, R. Baker, W. B. Davey, A. R. Guiblin, R. A. Jelley, A. J. Reeve, H. Routledge, F. Sternfeld, A. P. Wattt, M. S. Beer, D. N. Middlemiss, A. J. Noble, J. A. Stanton, K. Scholey, R. J. Hargreaves, B. Sohal, M. I. Graham, V. G. Matassa, J. Med. Chem., **38**, 1799-1810 (1995).

17. C. Y. Chen, D. R. Lieberman, R. D. Larsen, R. A. Reamer, T. R. Verhoeven, P. J. Reider, I. Cottrell, P. G. Houghton, Tet. Letters, **35(38)**, 6981-6984 (1994).

18. "Rizatriptan Benzoate," Drugs of the Future, **20(7)**, 676-679 (1995).

19. F. T. Noggle, C. R. Clark, K. H. Bouhadir, J. DeRuiter, J. Chromatogr. Sci, **29**, 31-36 (1991).

20. S. Vianna-Rodrigues, L. Martins-Viana, J. Quiroga, B. Insuasty, R. Abonia, W. Baumann, J. Heterocyclic Chem., **31**, 813-817 (1994).

21. R. Nowakowski, P. J. P. Cardot, A. W. Coleman, E.Villard, G. Guiochon, Anal. Chem., **67(2)**, 259-266 (1995).

22. N. Bargmann-Leyder, C. Sella, D. Bauer, A. Tambute, M. Caude, Anal. Chem., **67(5)**, 952-958 (1995).

23. K. Jinno, Y. Saito, Y. L.Chen, G. Luehr, J. Archer, J. C. Fetzer, W. R. Biggs, Journal of Microcolumn Separations, **5(2)**, 135-140 (1993).

24. Merck Research Laboratories materials synthesized for drug development.

25. Cerius^2 ™ Release 2.0, BIOSYM/Molecular Simulations, Cambridge, UK, 1995.

26. A. K. Rappé, C. J. Casewit, K. S. Colwell, W. A. Goddard, W. M. Skiff, J. Am. Chem. Soc., **114**, 10024 (1992).

27. A. K. Rappé, W. A. Goddard, J. Phys. Chem., **95**, 3219 (1991).

28. A. J. P. Martin, Biochem., Soc. Symp., **3(4)** (1949).

29. E. Grushka, H. Colin, G. Guiochon, J. Chromatogr., **248**, 325-339 (1982).

30. A. Tchapla, S. Heron, H. Colin, G. Guiochon, Anal. Chem., **60**, 1443-48 (1988).

31. F. M. Yamamoto, S. Rokushika, H. Hatano, J. Chromatogr. Sci., **27**, 704-709 (1989).

32. H. J. Issaq, M. Jaroniec, J. Liq. Chromatogr., **12**, 2067-2082 (1989).

33. A. Ishikawa, T. Shibata, J. Liq. Chromatogr., **12**, 2067-2082 (1989).

34. Y. Hatefi, W. G. Hanstein, Proc. Natl. Acad. Sci. USA, **62**, 1129 (1969).

35. D. Morel, J. Serpinet, J. Chromatogr., **200**, 95-104 (1980).

36. D. Morel, J. Serpinet, J. Chromatogr., **214**, 202-208 (1981).

37. D. Morel, J. Serpinet, J. Chromatogr., **248**, 231-240 (1982).

38. W. E. Hammers, P. B. A. Verschoor, J. Chromatogr., **282**, 41-58 (1983).

39. W. E. Hammers, J. C. Van Miltenburg, J. Chromatogr., **268**, 147-155 (1983).

40. D. Morel, J. Serpinet, G. Untz, Chromatographia, **18**, 611-614 (1984).

41. L. A. Cole, J. G. Dorsey, Anal. Chem., **64**, 1317-1323 (1992).

42. L. C. Sander, J. B. Callis, L. R. Field, Anal. Chem., **55**, 1068 (1983).

43. A. S. Shetty, J. Zhang, J. S. Moore, J. Am. Chem. Soc., **118**, 1019-1027 (1996).

44. MAC-MOD Zorbax SB-Phenyl column literature.

45. K. B. Sentell, J. G. Dorsey, J. Liq. Chromatogr., **11**, 1875-1885 (1988).

Received August 29, 1997
Accepted September 18, 1997
Manuscript 4609