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Concept Articles

Preparative Chromatography Technique in the Removal of Isostructural Genotoxic Impurity in Rizatriptan: Use of Physicochemical Descriptors of Solute and Adsorbent†

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Abstract:

The development of preparative negative hydrophobic interaction chromatographic technique based on molecular structural descriptors and physicochemical descriptors e.g. the dissociation constant (pK_a), partition coefficient (log*P*), and distribution coefficient (log*D*) for removal of a structurally related genotoxic dimer impurity from rizatriptan bulk drug is presented.

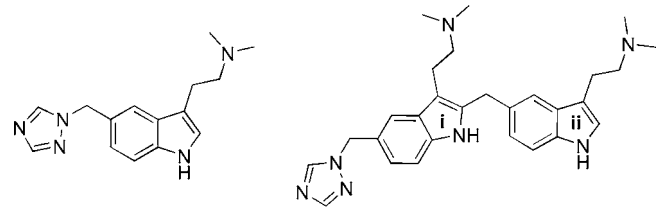

Figure 1. **Structure of rizatriptan (a) and dimer impurity (b).**

Introduction

Impurities can be generated at any stage of product development. Genotoxic and carcinogenic impurities tend to be less cumbersome in some cases, particularly where the drug products are cytotoxic and useful for cancer chemotherapy. In some cases, it is nearly impossible to eradicate impurities from drugs that impose a great challenge.

The limit of the genotoxic impurities should be reduced to "As Low As Reasonably Practicable" (ALARP) levels [1.5 μg/patient/day (after commercial launch)] that represent safe exposures for clinical applications.[1] For reducing these impurities to acceptable limits, there are various approaches that can be practiced. The most reasonable approach is to alter the synthetic process to control the level of impurities at the synthesis stage, or alternatively, the final product must be purified. The conventional methods for purification of active pharmaceutical ingredients (APIs) are fractional and repetitive crystallization and reverse- or normal-phase HPLC.

Preparative liquid chromatography has been used in the pharmaceutical industry since decades to purify low- and high-molecular weight molecules.[2] However, the high capital investment to acquire equipment does not make preparative HPLC a popular choice for unit operations.

In the synthesis of rizatriptan (Figure 1), a serotonin 5-HT (**1**) receptor agonist,[3] a genotoxic dimer impurity (Figure 1) was generated. This impurity could not be removed to an acceptable limit of mass fraction 0.01% by conventional processes such as fractional crystallization and recrystallization. Rizatriptan is FDA approved, and the impurity levels should be per the regulations. Herein, we present a low-pressure negative hydrophobic interaction liquid chromatography process approach which is based on molecular structural descriptors to remove the aforementioned genotoxic impurity by employing reusable polymeric adsorbents.

The quantitative structure retention relationship (QSRR) model-based strategy for property prediction of a drug has been in practice since the 1970s. This structure-based approach was used for the prediction of pK_a, log *P* and log *D* of rizatriptan and the dimer impurity using demo version of computational software Pallas (CompuDrug International Inc. South San Francisco, CA, U.S.A.).

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(1) *Regulation and Control of Genotoxic Impurities in Pharmaceuticals*; 2nd Annual Non-Clinical Conference of the Drug Information Association (DIA), Bethesda, Maryland, U.S.A., November 17−18, 2005; http://www.diahome.org.

(2) (a) Rathore, A., Velayudhan, A., Eds.; *Scale Up and Optimization in Preparative Chromatography, Principles and Biopharmaceutical Applications*,; Chromatographic Science Series, Vol. 88; Mercel Dekker Inc.: New York, 2003; pp 289−316. (b) Cox, G. B., Ed.; *Preparative Enantioselective Chromatography*; Blackwell Publishing: Oxford, 2005; pp 297−302.

(3) Dahlof, C. G. H.; Rapopoti, A. M.; Sheftell, F. D.; Lines, C. R. *Clin. Ther.* **1999**, *21*, 1823.

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Table 1. **Predicted values of pK_a and log *P* of rizatriptan and dimer impurity**

	pK_a for acidic group (s)	pK_a for basic(s)		
cmpd	pyrrole (acidic)	$RCH_2N^+H(CH_3)_2$	1,2,4-triazole	log*P*
rizatriptan	15.70	9.94	3.09	2.59
dimer impurity	(i) 15.71, (ii) 16.42	(i) 9.87, (ii) 9.94	3.09	4.94

In particular, the concept relies on the relationship between certain molecular descriptors and functions of the selected standard stationary phases belong to the molecule.[4] The molecular structural descriptors used by QSRR approach in reverse-phase LC are hydrophobicity, log *P*, and hydrophobicity substitution constant, π. Other descriptors used have been geometrical descriptors such as van der Waals volume (Vw), van der Waals surface area (Aw), and shape parameter or length-to-breadth ratio (*L*/*B*); topological descriptors such as connectivity index χ, and correlation factor (*F*); and electronic descriptors such as Hammett's constant, σ, and HD and HA which are proton-donating and proton-accepting properties, respectively.[5,6]

These predicted, or experimentally determined, molecular descriptors of an analyte have been used for prediction of chromatographic retention behavior or elution behavior of proteins, peptides,[7−10] and small molecules such as synthetic or natural products.[7,11−17] The proposed model has extensively been used to understand the retention mechanism for development of analytical LC methods. One of the important structure-based physicochemical molecular descriptors in LC separations is hydrophobicity, whereas the other dominant descriptors are the shape and size of molecules for nonpolar compounds, and electronic descriptors for polar compounds.[6] Hydrophobicity is known to be the most important factor for retention characteristics in reverse-phase chromatography (RPC) and hydrophobic interaction chromatography (HIC).

Retention differences related to chemical structure have been evaluated on the basis of molecular interactions related to solubility of a solute. Relative solubility of solutes can be expressed in terms of the octanol/water partition coefficient called log *P* is proposed mathematically as Hansch's π-constant (describes the contribution of a substituent to the lipophilicity of a molecule), and later incorporated in Rekker's hydrophobic fragmental constant (describes the contribution of a substituent to the hydrophobicity of a molecule). However, Hansch's π-constants and Rekker's hydrophobic fragmental constants are relative values and do not explain the full implications of solute properties, especially those that have a tendency to dissociate in solutions.[18] Thus, although log *P* is used as an index of hydrophobicity in many studies,[19] this describes hydrophobicity of only the neutral form of the solute.[20] Therefore, further refinement of retention models was attempted using another convenient measure of the analyte hydrophobicity and physicochemical property: log *D*, which is essentially the logarithm of the distribution coefficient of the analyte between octanol and water phases, accounting for both ionic and nonionic forms of solute.[20] Inclusion of the acid dissociation constant (pK_a) in the calculation makes it possible to predict the retention behavior of ionized compounds. The partition coefficient calculated by Rekker's method has been shown to have the following linear relationship with log k', where k' is the retention factor in RPC.[18,19]

$$\log k' = y \log P + m \tag{1}$$

where *y* and *m* are the slope and intercept of an experimental calibration curve. In the same manner a more general equation based on log *D* can be written as

$$\log k' = y \log D + m \tag{2}$$

As a result, resolution between two solutes can be estimated from Δlog *D* which is difference between log *D* values of the solutes under specified conditions.

Acid dissociation constant (pK_a), partition coefficient (log *P*), and distribution coefficient (log *D*) are important physicochemical descriptors related to the structure of a molecule. These parameters were predicted from the chemical structures of rizatriptan and its dimer impurity. The prediction reveals that rizatriptan exhibits three pK_a's, whereas the dimer impurity has five pK_a values (Table 1).

In a classical paper entitled "Adsorption separation by salting out", Tiselius[30] laid down the foundation for a separation method popularly known as hydrophobic interaction chromatography (HIC). Many theories proposed for HIC are based upon those derived for interactions between hydrophobic solutes and water.[22] What is common to all is the central role played by the structure-forming salts and the effects they exert on solute, solvent, and the adsorbent of the chromatographic system to

(4) (a) Kaliszan, R. *Quantitative Structure-Chromatographic Retention Relationship*; Wiley Interscience: New York 1987. (b) Soares, M. d. F.; Monache, F. D.; Heinzen, V. E. F.; Yunes, R. A. *J. Braz. Chem. Soc.* **1999**, *10*, 189.
(5) Schnackenberg, L. K.; Beger, R. D. *J. Chem. Inf. Model.* **2005**, *45*, 360.
(6) Jinno, K. *Chromatography* **2001**, *22*, 1.
(7) Barbosa, J.; Toro, I.; Berges, R.; Sanz-Nebot, V. *J. Chromatogr., A* **2001**, *915*, 85.
(8) Song, M.; Breneman, C. M.; Bi, J.; Sukumar, N.; Bennett, K. P.; Cramer, S.; Tugcu, N. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 1347.
(9) Mahn, A.; Asenjo, J. *A. Biotech. Adv.* **2005**, *23*, 359.
(10) Ladiwala, A.; Rege, K.; Breneman, C. M.; Cramer, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 11710.
(11) Yamamoto, F. M.; Rokushika, S.; Hatano, H. *J. Chromatogr.* **1987**, *408*, 21.
(12) Roses, M.; Bolliet, D.; Poole, C. F. *J. Chromatogr., A* **1998**, *829*, 29.
(13) Sanli, N.; Fonrodona, G.; Barron, D.; Ozkan, G.; Barbosa, J. *J. Chromatogr., A* **2002**, *975*, 299.
(14) Baczek, T.; Kaliszan, R. *J. Chromatogr. A* **2003**, *987*, 29.
(15) Lee, S. K.; Row, K. H. *Bull. Korean Chem. Soc.* **2004**, *24*, 1265.
(16) Baeza-Baeza, J. J.; García-Alvarez-Coque, M. C. *Anal. Chim. Acta* **2004**, *521*, 61.
(17) Subirats, X.; Bosch, E.; Roses, M. *J. Chroamtogr., A* **2006**, *1121*, 170.
(18) Hanai, T. *J. Chromatogr.* **1991**, *550*, 313.
(19) Funasaki, N.; Hada, S.; Neya, S. *J. Chromatogr.* **1986**, *361*, 33.
(20) (a) MeBrien, M. A. KolovanovE.; Tashlitsky, V. CPSA, Princeton, NJ USA (2004) http://www. acdlabs.com. (b) Csizmzdiz, F.; Tsantili, K.; Anna, P. I.; Darvas, F. *J. Pharm. Sci.* **1997**, *86*, 865. (c) Csizmzdiz, F.; Tsantili, K.; Anna, P. I.; Darvas, F. *J. Pharm. Sci.* **1997**, *86*, 1173.
(21) Porath, J.; Sundberg, L.; Fornstedt, N.; Olson, I. *Nature* **1973**, *245*, 465.
(22) Tanfon, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membrane*; John Wiley and Sons: New York, 1973.

Table 2. **Properties of adsorbent matrices**

properties of referential lot	SEPABEADS SP207	DIAION HP2MG	SEPABEADS SP700
type	modified polystyrenic	methacrylic	polystyrenic
surface area (m^2/g)	600	470	1200
pore radius (nm)	12.1	19.8	8.2
pore volume (mL/g)	1.1	1.2	2.3
average particle diameter (μm)	460	490	529
solubility index	10.7	8.4	9.7
apparent density (g/L)	789	732	691

bring about the binding of solute and adsorbent. In view of this, Porath[31] proposed 'salt-promoted adsorption'; Hofstee[32] and Shaltitel[33] proposed self-association of molecules in water; Hajerten[34] proposed displacement of ordered water surrounding hydrophobic groups of solute and adsorbent, leading to increased entropy; Melander and Horvath[35] proposed increase in surface tension of water arising from structure-forming salts dissolved in it; and finally Srinivasan and Ruckenstein[36] have proposed van der Waals attraction forces between protein and ligands. The main parameters to consider when selecting HIC media and the optimizing separation process are matrix properties (such as solubility index, base matrix, particle size, pore radius, surface area, etc.), type and concentration of salt, the pH, temperature, and additives.

There are scant reports indicating the use of these physicochemical molecular descriptors for the prediction of retention, development, and optimization of preparative LC for industrial production of active pharmaceutical ingredients (API).[4b] In the present work three solute molecular descriptors pK_a, log *P*, log *D* along with buffered mobile phase descriptors pH and ionic strength were used to develop a preparative hydrophobic LC method for removal of the above said impurity from rizatriptan.

The crude mixture containing mass concentrations of 70% rizatriptan and 4% structurally related dimer impurity, rizatriptan benzoate, and the dimer impurity standards were obtained from Dr. Reddy's Laboratories Ltd. (Bollaram, AP, India). The rigid, porous, synthetic polymer-based SEPABEADS and DIAION adsorbents (Table 2) are from Resindion s.r.l., (Mitsubishi Chemical Corporation, Binasco, Italy). Fundamental characteristics of these adsorbents have been described elsewhere.[28a] All other chemicals and solvents were of synthesis grade except those for HPLC analysis, where analytical grade chemicals and solvents were used and were purchased from Merck, India. Demineralized water was prepared by Milli-Q system (Millipore, Bedford, MA, U.S.A.).

Materials and Methods

Preparative Chromatography System. All preparative chromatography experiments were performed with a BioRad Biologic Duoflow pathfinder system (BioRad Laboratories, Hercules, CA, U.S.A.) consisting of workstation, maximizer, AVR3-7 auto injector valve, SVT3-2 valve, QuadTec UV−vis detector, conductivity monitor, pH monitor, and ECONO pump for external loading. Data acquisition was performed by using Biologic Duoflow software, and a Biologic Biofrac fraction collector was used for fraction collection.

Analytical Chromatography System. Analyses of fractions obtained from each experiment were performed with gradient HPLC (Jasco, Tokyo, Japan) consisting of two PU-2080 HPLC pumps, UV-2070 UV−visible detector, and 851-AS auto sampler. Data acquisition and chromatograms processed for determination of retention times (RT), peak area, and signal-to-noise (*S*/*N*) ratio were done by using Borwin chromatography software (Jasco, Tokyo, Japan).

Analytical Method for Determination of Rizatriptan and Dimer Impurity. The analytical method comprises mobile phase A that was prepared by dissolving 6.8 g of KH_2PO_4 (Merck Ltd., Mumbai, India) in 1 L of Milli-Q water to which was added 1.5 mL of triethylamine (TEA); the pH was adjusted to 4.0 with dilute H_3PO_4 (Merck Ltd., Mumbai, India),[28b] and the solution was filtered through 0.45 μm Millipore filter. Mobile phase B was prepared by mixing methanol with water in 1:1 volume ration. An ACE-cyano analytical chromatography column (250 mm × 4.6 mm, 5 μm) was obtained from Advanced Chromatography Technologies (ACE, Aberdeen, Scotland). HPLC analysis was carried out at 0.8 mL/min flow rate, ambient temperature, 20 μL sample injection, and detector equipped at 210 nm wavelength. Isocratic flow of 7% mobile phase B in A for 11 min followed by a gradient from 7% to 40% of mobile phase B in A in 30 min was used. The dimer impurity has a relative response factor (RRF) of 1.51 and elutes at relative retention time (RRT) of 2.3 with respect to the rizatriptan retention time. The standard curve of rizatriptan benzoate was prepared in the range of 0.2−1.5 mg/mL concentration, and the content of rizatriptan in the fraction obtained from preparative chromatography was determined from the equation of the standard curve. Content of dimer impurity was reported as % area on HPLC with respect to the rizatriptan peak.

(23) Hijerten, S. *J. Chromatogr.* **1973**, *87*, 325.
(24) Hijerten, S.; Yao, K.; Eriksson, K. O.; Johnsson, B. *J. Chromatogr.* **1986**, *359*, 99.
(25) Barbosa, J.; Berges, R.; Sanz-Nebot, V.; Toro, I. *Anal. Chim. Acta* **1999**, *389*, 43.
(26) Heinisch, S.; Rocca, J. L. *J. Chromatogr., A* **2004**, *1048*, 183.
(27) *DIAION Manuals I and II*; Mitsubishi Chemical Corporation: Tokyo, Japan.
(28) (a) Adachi, T.; Isobe, E. *J. Chromatogr., A* **2004**, *1036*, 33. (b) Antonucci, V.; Wright, L.; Toma, P. *J. Liq. Chromatogr. Relat. Technol.* **1998**, *21*, 1649.
(29) Kaliszan, R. *J. Chromatogr., A* **1993**, *656*, 417.
(30) Tiselius, A. *Arh. Kem.* **1948**, *26B*, 1.
(31) Poath, J. *J. Chromatogr.* **1986**, *376*, 331.
(32) Hofstee, B. H. *Anal. Biochem.* **1973**, *52*, 430.
(33) Shaltiel, S.; Er-el, Z. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 778.
(34) Hjerten, S. *Proceedings International Workshop on Technology for Protein Separation and Improvement of Blood Plasma Fractionation*; U.S. Government Printing Office: Washington, DC, 1978; International Workshop on Technology for Protein Separation and Improvement of Blood Plasma Fractionation, Reston, VA,September 7−9, 1977; National Heart, Lung, and Blood Institute. DHEW publication no. 78-1422; 1978; p 410.
(35) Melander, W.; Horvath, C. *Arch. Biochem. Biophys.* **1977**, *183*, 200.
(36) Srinivasan, R.; Ruckenstein, E. *Sep. Purif. Methods* **1980**, *9*, 267.

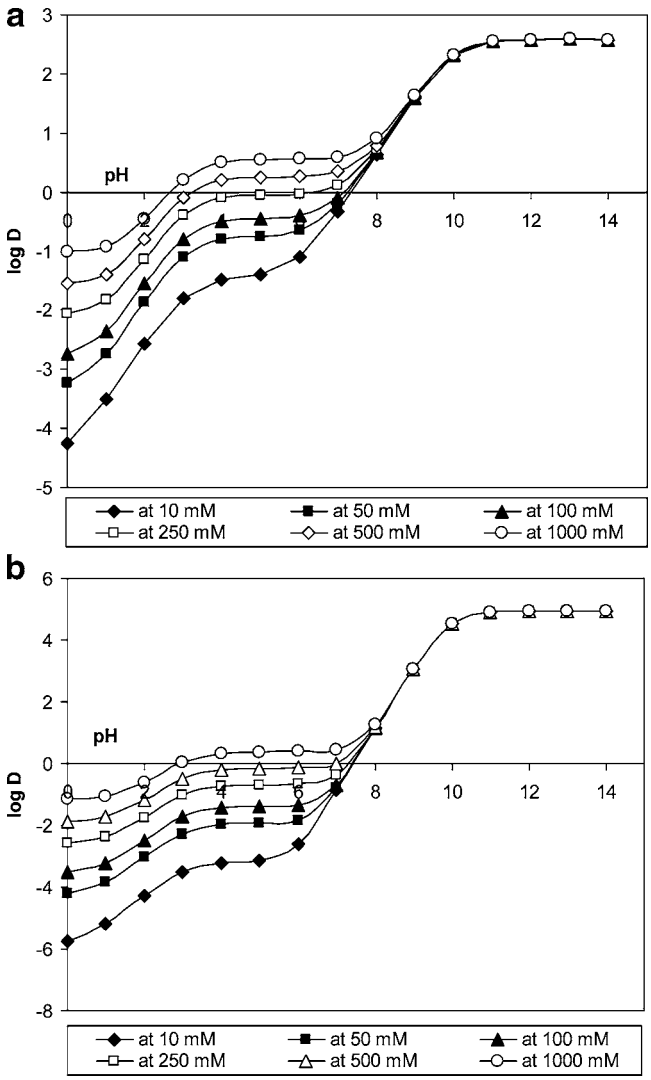

Figure 2. **(a) Characteristic plot of predicted log *D* values of rizatriptan against pH at different ionic strengths in aqueous buffer system. (b) Characteristic plot of predicted log *D* values of dimer impurity of rizatriptan against pH at different ionic strengths in aqueous buffer system.**

Prediction of pK_a, log *P*, and log *D* for Rizatriptan and Dimer Impurity. Prediction of pK_a, log *P* and log *D* for rizatriptan and dimer impurity (Table 2) was achieved using Pallas model software that utilizes the structure-based approach. Log *D* was also predicted at different pH and ionic strength conditions (plots a and b of Figure 2) and used for calculation of Δlog *D* (eq 3). These data were used to select the initial conditions for development of chromatographic process to remove dimer impurity from rizatriptan.

Preparation of Buffers for Preparative Chromatography. For optimization of loading on matrix, aqueous solutions of different ionic strengths were prepared using KH_2PO_4 and K_2HPO_4 salts. Depending upon prediction of log*D* pattern at various pH and ionic strength acidic and basic aqueous salt solutions were prepared by adjusting pH using orthophosphoric acid and sodium hydroxide accordingly to determine actual retention behavior of rizatriptan and dimer impurity on adsorbents. For all chromatographic trials, fresh buffers were made daily using Milli Q water. These buffers were used for equilibration and washing of column before and after loading of crude reaction mixture.

Column Experiments. All column experiments were performed on the BioRad Biologic Duoflow system using 25 mm $\times$ 100 mm and 50 mm $\times$ 300 mm I.D. glass column having presealed distributor frit at the bottom and equipped with one 140 mm long BioRad ECONO adjustable flow adaptor with porous polymeric distributor. The top adaptor was connected to AVR7-3 auto injector valve to which external loading ECONO pump was connected through one inlet of SVT3-2 valve. Another inlet of SVT3-2 valve was connected to workstation pump. Bottom outlet of column was connected to QuadTec UV/vis detector equipped at 210 nm wavelength. Column experiments were performed on SEPABEADS and DIAION adsorbents with bed height of 80 mm and 250 mm for column of 25 mm and 50 mm I.D. respectively. Separate runs were made for different ionic strength and pH condition. Operating steps such as equilibration, loading, washing and water flushing was performed in *down flow* mode where as regeneration was carried out in *up flow* mode. All steps were carried out at 100 cm/h linear velocity for all experiments. Different pH and ionic strengths conditions for equilibration, loading and washing were evaluated to optimize the desired separation of rizatriptan from reaction mixture.

Reaction mixture obtained after synthesis was highly acidic and was quenched to desired pH with 10% (w/v) sodium hydroxide solution before loading to the column. The pH and ionic strength of aqueous based equilibration and washing solutions was varied from one experiment to another.

Result and Discussions

Selection of pH and Ionic Strength from Predicted pK_a, log *P* and log *D* of Rizatriptan and Dimer Impurity. In both molecules, the pyrrole ring in benzimidazole nucleus shows acidic whereas 1,2,4-triazole and dimethlyamino groups show basic characters. Further, it was observed that the log *P* value for rizatriptan is less than that of the impurity and indicates that at neutral conditions of the molecules, dimer impurity is more hydrophobic than rizatriptan. Achieving the neutral conditions of two different molecules in the same environment is difficult, as the hydrophobicity of a molecule is governed by the pH. Instead useful molecular descriptors for predicting the retention behavior of a molecule in reverse-phase or hydrophobic interaction chromatography are log *P* and log *D* that describe the ionization pattern of a molecule.

The changes of log *D* of rizatriptan and its dimer were plotted against the pH and ionic strength of the aqueous mobile phase to give a characteristic plot as shown in a and b or Figure 2, respectively. The plots in Figure 2 indicate that both the rizatriptan and dimer impurity are basic in nature. Log *D* values increase with pH, because in the case of basic compounds the number of ionized species (or total overall charge on the molecule) decreases with an increase in pH. This makes both molecules increasingly nonpolar or hydrophobic towards a more basic environment. Characteristic changes between log *D* values of rizatriptan and dimer impurity were also observed. Below pH 8, log *D* values of rizatriptan are higher than those of the impurity, which is due to the presence of three basic groups on the dimer impurity, making it more polar compared to rizatriptan

having only two ionization groups. This indicates that, below pH 8, rizatriptan is more hydrophobic than the impurity.

Solute-adsorbent interactions were strongly affected by the ionic strength and pH of the mobile phase in hydrophobic interaction chromatography.[21−24] Therefore, the effects of ionic strength and pH on retention[7,17,25,26] behavior in terms of log *D* for both rizatriptan and dimer impurity were determined.

In an aqueous acidic environment, the log *D* of the dimer impurity was less than the log *D* of rizatriptan. Also the log *D* values of rizatriptan and the dimer impurity increase with increased salt concentration. The Δlog *D* values at different ionic strength were calculated from the log *D* values of rizatriptan and its dimer impurity as follows:

$$\Delta \log D = \log D_{\text{riz}} - \log D_{\text{dimer}} \qquad (3)$$

Further, it was revealed that not only the molecular descriptors of solute but also the adsorbent properties [hereafter termed as adsorbent descriptors (such as thermodynamic descriptors: solubility index and chemical surface and hydrodynamic descriptors: surface area, pore radius, and pore volume)][27] should be considered for the development of preparative LC processes. Therefore, in the present work adsorbents having variations in their solubility index, surface area, pore radius, and pore volume were also studied for desired degree of separation.

The combined effects of pH and salt concentration on log *D* and adsorbent descriptors were used for design and development of hydrophobic interaction chromatography, where rizatriptan was recovered in unbound and wash fractions, resulting in a negative purification process for product recovery. The dimer impurity remains bound to the matrix under loading and washing conditions and was cleaned by using organic solvent during regeneration of the matrix. Thus, the structure-based approach used for prediction of log *D* has reduced optimization work time and helped to use the highest possible loadability of the matrix to afford a better production rate.

Further, it was observed that, from pH 8 to 10, the log *D* value of the dimer increases suddenly and becomes greater than the log*D* value of rizatriptan. Above pH 11 log *D* values for both compounds remain steady. This is due to suppression of ionization of both molecules at pHs at and above pH 8, which decreases the total charge on the molecule, leading to increased log *D* values. Above pH 11, both molecules become almost neutral, and no changes have been observed in log *D* values with respect to pH. Here, dimer impurity (MW 485.67) exhibits higher log*D* values than rizatriptan (MW 269), because hydrophobicity of a solute is determined by molecular size and bulkiness, which are reliable descriptors of dispersive interactions when polar interactions are negligible or constant.[29] Plots a and b of Figure 2 also show that there is an increase in log *D* values with an increase in salt concentration, which indicates that the molecules become more hydrophobic at elevated ionic strength. This is due to the fact that salt ions form the a complex with oppositely charged species of the molecule leading to a decreased overall charge on the molecule, which in turn increases the hydrophobicity (i.e., log *D*). Therefore, at a particular pH, when salt concentration increases, more and more ionic species of the molecules become neutral and hence

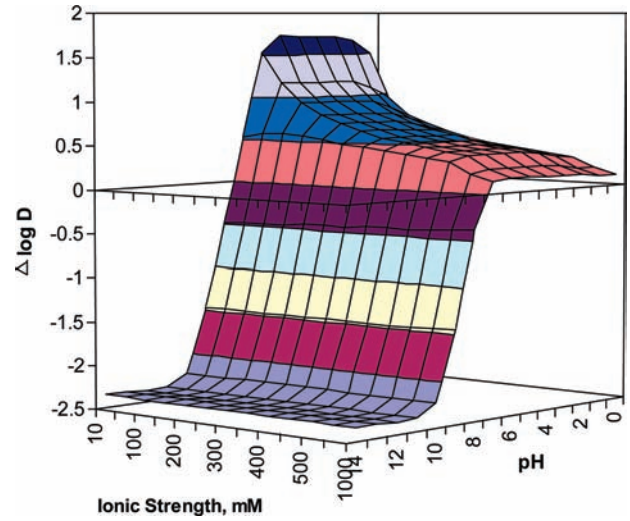

Figure 3. **Effect of ionic strength and pH on Δlog *D*.**

increasingly hydrophobic. This salt- and pH-dependent hydrophobic behavior of rizatriptan and dimer impurity can be used to develop the chromatographic separation and also to increase the production rate by utilizing the maximum possible capacity or loadability of the adsorbent matrix.

Figure 3 shows effects of salt concentration and pH on Δlog *D* (eq 3), where Δlog *D* was considered as a difference between log *D* of the molecule of interest (rizatriptan) and the impurity (dimer) to be removed. It is observed that below pH 8, salt concentration has significant effect on Δlog *D*, which decreases and approaches to zero with an increase in salt concentration. This reveals that at high salt concentration, separation (or resolution) of rizatriptan and dimer impurity is difficult as both molecules will try to remain in the same hydrophobic environment (i.e., stationary phase), leading to loss of selectivity. Thus, selectivity of adsorption decreases at elevated ionic strength under acidic conditions, where Δlog *D* approaches zero. However, the presence of low salt concentration is necessary for the differential retention of rizatriptan and dimer impurity which otherwise remain unadsorbed and appear in the void volume due to the highly polar nature of both molecules under acidic environment. At pH 8, the sign of Δlog *D* changes, indicating that the hydrophobic behaviors of rizatriptan and dimer alter; the dimer becomes more hydrophobic after pH 8, which is initially less hydrophobic (below pH 8). This reveals that pH 8 is important and separation would be possible at proper ionic strength and that at the same time loadability can be increased by increasing the ionic strength, which in turn increases the productivity. Thus, pH 8 was found to be critical where altered hydrophobic behavior was observed.

Column Adsorption: pH-Based Selection of Adsorbent Matrix for Removal of Dimer Impurity. It is known that for compounds having higher pK_a values, the retention increases with increase in pH.[7] This could be explained due to the equilibrium between charged and neutral forms. Therefore, depending upon the effect of pH[26] and log *D*,[18] on retention, two possibilities were tried: (1) allow rizatriptan to bind selectively keeping the dimer in unadsorbed fractions at pHs 3 and 6; (2) allow the dimer to bind selectively on the resin and keep rizatriptan in unadsorbed fractions at pHs 8 and 9 (pH greater than 9 was avoided due to solubility limitation of the

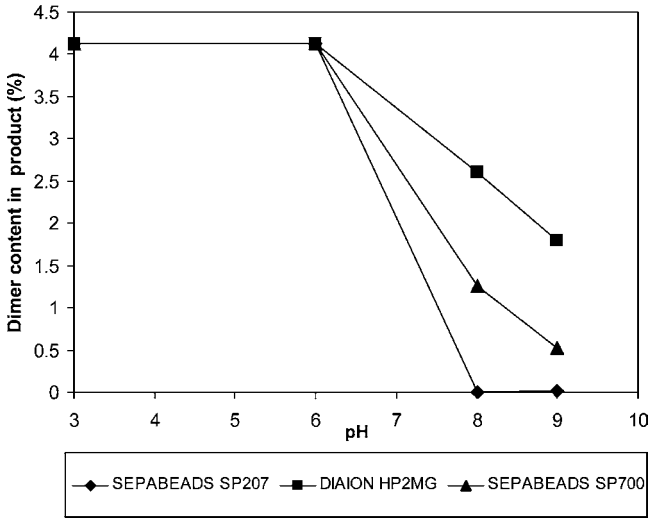

Figure 4. **Dimer content (%) in rizatriptan recovered in unbound and wash fractions; (Load: 2 g of rizatriptan containing 4.12% dimer impurity, equilibration and washing with aqueous solution of phosphate with 50 mM ionic strength at respective pHs).**

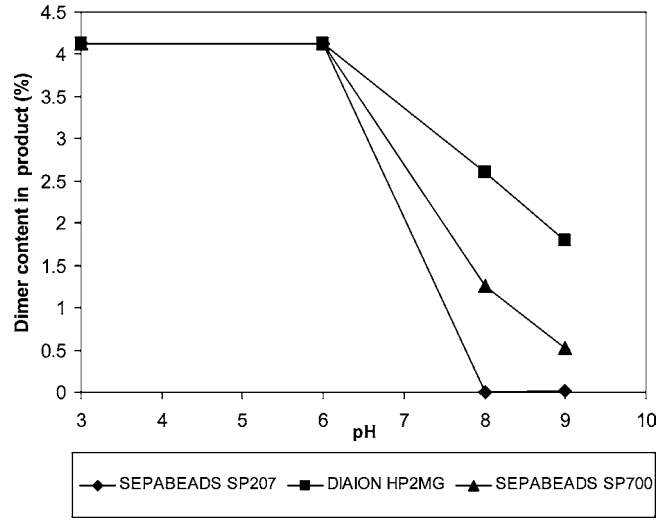

Figure 5. **Optimization of loadability (g/L) and productivity (g/L/h) for rizatriptan, having dimer impurity below the acceptable limits on SEPABEADS SP207 adsorbent.**

product in aqueous environment). In the former possibility, experiment was carried out on different adsorbents by loading a quenched reaction mixture containing 2 g of rizatriptan and 4.12 (%) of dimer impurity in 25 mm I.D. column. Results obtained are shown in Figure 4, which indicates no reduction in dimer impurity was observed for all three adsorbents. Both dimer and product remained significantly unadsorbed and appeared in flow-through and wash fractions. This nonretaining behavior might be due to the highly polar molecular species of both rizatriptan and dimer molecules, which have log*D* values of less than −0.65 (a and b of Figure 2), below pH 8. Further it was concluded that, even though binding will occur at higher ionic strength, it may result in decreased selectivity and hence loadability due to Δlog*D*, which approaches zero at elevated ionic strength under aqueous acidic conditions (Figure 3).

However, when loading was carried out at pH 8, using 50 mM ionic strength solution for equilibration and washing, it was seen that rizatriptan was obtained in unadsorbed and wash fraction with substantial reduction of dimer content (Figure 4) relative to rizatriptan. The highest possible reduction in dimer impurity (0.001%) was observed with SEPABEDS SP207. The dimer impurity remained adsorbed in the column due to its higher log *D* (1.13) at pH 8, which makes it more hydrophobic than rizatriptan having log *D* of 0.66. Thus, rizatriptan has a preferred polar aqueous environment at pH 8 and 50 mM ionic strength, where selective adsorption of the dimer on adsorbent occurred. Here, 98% rizatriptan was recovered with 4120-fold reduction in dimer impurity.

Further, an experiment was carried out at pH 9 and 50 mM ionic strength, where 85% product was recovered containing 0.02% of dimer impurity from SEPABEADS SP207 adsorbent matrix. Low recovery and leakage of dimer impurity occurred in the product containing fractions due to higher log *D* values at pH 9, which makes both molecules more hydrophobic; hence, the stronger hydrophobic interaction of even rizatriptan with the SEPABEADS SP207 makes less effective surface area available for adsorption of dimer impurity.

Both SEPABEADS SP700 and DIAION HP2MG have resulted in unsatisfactory reduction of the dimer impurity (more than 0.01%) in the recovered product fraction at pHs 8 and 9. This shows that not only do the physicochemical descriptors of an analyte play an important role in selectivity and resolution in chromatographic separations but so does the adsorbent. The important adsorbent descriptor, solubility index, is a measure of the hydrophobicity, and accordingly, an adsorbent with a larger solubility index (Table 1) has a higher adsorption strength.[27] Therefore, SEPABEADS SP207 having a solubility index of 10.7 showed stronger retention of dimer impurity than did SPABEADS SP700 and DIAION HP2MG, having solubility indexes of 9.7 and 8.4, respectively.

Another important finding was that, even though the SEPABEADS SP700 has higher surface area and pore volume (Table 1) than does SEPABEADS SP207, low selectivity is a result of the small pore radii. Also, in the case of DIAION HP2MG, the surface area and pore volume were less because of the larger pore radius than those of both SEPABEADS SP207 and SP700 and has resulted in even lower selectivity for dimer removal. Here, the former case shows the effect of the solubility index; whereas the latter case shows the effect of the solubility index as well as the surface area[28] on reduction of dimer impurity under aqueous experimental conditions.

Increasing the Loadability to Improve Productivity: Ionic Strength-Based Approach. From Figure 3, it is observed that, at pH 8, there was no significant effect of ionic strength on Δlog *D*, but log *D* of both molecules increases with increase in salt concentration as shown in a and b of Figure 2. This reveals that *loadability* can be increased without affecting selectivity at increased ionic strength. Figure 5 shows results of studies carried out on 500 mL of adsorbent matrix in a 50 mm × 300 mm BioRad ECONO glass column, at 50 mM to 750 mM ionic strength, with increased loading from 2−8 g of rizatriptan in the reaction mixture. In this negative hydrophobic interaction chromatography, *loadability* was defined as the quantity of rizatriptan that could be loaded on the adsorbent matrix leading to greater than 95% rizatriptan recovery in unbound and wash fractions with dimer impurity below the

Table 3. **Reuse of resin column after CIP**

entry	% of **a** (before elution)	% of **b** (before elution)	% of **a** (after elution)	% of **b** (after elution)	% of **a** (after CIP)	% of **b** (after CIP)
1	79.9	3.7	86.2	0.007	ND	ND
2	81.6	3.5	91.1	0.008	ND	ND
3	**84.0**	**3.4**	**93.7**	**ND**	**ND**	**ND**
4	79.1	4.0	92.4	0.004	ND	ND

acceptable limit. It was found that *loadability* and *productivity* were increased from 50 g/L to 200 g/L and 12.5 g/L/h to 33 g/L/h, respectively, when the ionic strength was increased from 50 mM to 250 mM, with dimer impurity undetectable by HPLC. This may be attributed by increase in log *D* with increased ionic strength as well as selective and stronger retention[18] of dimer impurity on higher surface area for adsorption available due to non-retaining rizatriptan.

Further, an increase in ionic strength has shown a deleterious effect on loadability and productivity, which was restricted by dimer impurity concentration (which is about 4% w/v in the feed reaction mixture) and log *D* of rizatriptan. Higher log *D* of rizatriptan (0.79) at 500 mM ionic strength was found to be sufficient to enhance the hydrophobic interaction between rizatriptan and matrix, where rizatriptan also starts binding (weakly) to the matrix, making less surface area available for dimer impurity adsorption. At 750 mM ionic strength, when 8 g of rizatriptan in reaction mixture was loaded, no rizatriptan was recovered in unbound and wash fractions, indicating strong retention of both molecules on the adsorbent matrix.

Thus, equilibration and washing with an aqueous solution of 250 mM ionic strength and pH 8 gave the highest possible loadability and productivity without breaching the dimer impurity, and these were chosen as the optimum conditions for the process.

Development of Cleaning-In-Place (CIP) Protocol for Reuse of Resin Column. After each cycle, bound substances (i.e., dimer impurity and other components of reaction mixture) must be washed out from the column to restore the original function of the medium. Also, the reproducibility and performance of the process under optimized conditions determines the process economics of the resin column reused after regeneration.[2] Therefore, different solutions were investigated for their ability to regenerate the resin column. Initial testing was done with pure methanol, acetonitrile and acetone. Due to possibility of retention of residual dimer impurity on adsorbent, mixtures of small amount of water (with acid and base modifiers) in methanol and acetonitrile were investigated. The optimum regenerating solution was found to be 95:5 methanol/water with 0.05% of ammonia. HPLC results show that after 5 bed volumes of this solution, dimer content of effluent was less than 1 ppm or not detected (Table 3, entry 3). Lab experiments indicated that the column could be regenerated and reused without infiltratration of the dimer impurity in the product.

Four consecutive trials have been taken after regenerating the matrix which show that greater than 95% rizatriptan was recovered as shown in Table 3.

Conclusions

We have shown herein that removal of structurally related impurities from synthetic API using low-pressure preparative chromatography on commercially available adsorbents has great potential in liquid chromatography. Retention in hydrophobic and reverse-phase chromatography is based on hydrophobicity and charge differences of product and impurities. Therefore, specific separation problems may be solved by predicting physicochemical descriptors of solute and applying them to understand the selectivity and retention mechanism as well. Further, the physicochemical descriptors of adsorbent such as solubility index, surface area, particle size, and pore radius may also play important roles in designing and optimizing the desired degree of selective separation and may be correlated with molecular descriptors of solute. Also the effective selection of mobile phase pH and ionic strength based on the structures of known compounds can speed up the process of method development and improve the robustness of the resulting method.

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