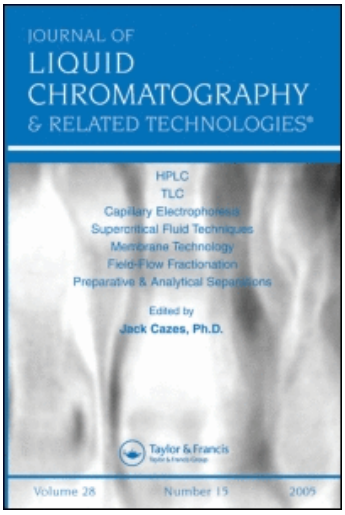

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HPLC Separation of Selected Cardiovascular Agents on Underivatized Silica Using an Aqueous Organic Mobile Phase

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HPLC SEPARATION OF SELECTED CARDIOVASCULAR AGENTS ON UNDERIVATIZED SILICA USING AN AQUEOUS ORGANIC MOBILE PHASE

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ABSTRACT

High performance chromatographic separations of selected cardiovascular agents (propranolol, atenolol, metoprolol, verapamil, diltiazem, nifedipine, clonidine and prazosin) on underivatized silica using aqueous phosphate buffer - acetonitrile mobile phases were studied. Mobile phases differing in organic modifier concentration, ionic strength and buffer pH were prepared and tested for chromatographic separation of selected mixtures of the analytes grouped according to pharmacological activity. The best separations of all analytes were obtained using a mobile phase of 60:40 v/v aqueous pH 3 phosphate buffer - acetonitrile at a 1.0 mL/min flow rate. Substitution of methanol for acetonitrile gave increased retention of the analytes with some reduction in column efficiency measured as plate counts. Although ion-pairing appears to be the primary interactive force between the silica column and the analytes, other forces such as hydrogen bonding and hydrophobic interactions are also involved in the separation.

INTRODUCTION

Reversed-phase high performance liquid chromatography (RP-HPLC) on bonded stationary phases has developed into a major analytical tool for separation and quantitation of most pharmaceuticals. However, reports have appeared in the scientific literature describing the excellent separation of basic (1-8), acidic (9,10), and neutral drugs (11-13) on underivatized silica using aqueous organic mobile phases. These systems showed clear improvement in peak shape, plate counts and efficiency as compared to conventional bonded phase chromatography. The predominant retention mechanism for basic compounds was found to be cation exchange with silanol groups on the silica surface. A mixed retention mechanism of hydrogen bonding and quasi-reversed-phase retention was reported for acidic drugs. Neutral drugs were determined to be retained on silica by hydrogen bonding or other non-specific forces.

This study was designed to investigate the applicability of underivatized silica to the analysis of cardiovascular agents. Model compounds were selected from three classes, including β-adrenergic blockers, calcium channel blockers, and α-adrenergic blockers, which are medically important and widely used. Chromatographic separations for these cardiovascular drugs were developed on underivatized silica using an aqueous buffer- acetonitrile mobile phase.

EXPERIMENTAL

Reagents and Chemicals

The structural formulae of the compounds studied are shown in Fig 1. Propranolol HCl was a gift from Ayerst Laboratories Inc. (New York, New York, U.S.A.). Atenolol was obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). Metoprolol was a gift from CIBA-Geigy Pharmaceutical Company (Summit, NJ, U.S.A.). Nifedipine and Prazosin HCl were obtained from Pfizer Inc. (Brooklyn, NY, U.S.A.). Verapamil HCl was a gift from Knoll Pharmaceutical Company (Whippany, NJ, U.S.A.). Diltiazem HCl and Clonidine HCl were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acetonitrile and water were HPLC grade (J.T. Baker, Phillipsburg, NJ, U.S.A.) Monobasic sodium phosphate, sodium hydroxide and concentrated phosphoric acid were also obtained from Baker.

Instrumentation

Chromatography was performed on an HPLC system consisting of a Beckman Model 110B HPLC pump (Fullerton, CA, U.S.A.), a Rheodyne Model 7125 injector equipped with a 50-μL loop (Cotati, CA, U.S.A.), and a Micromeritics Model 787 variable UV/VIS detector (Norcross, GA, U.S.A.). The analytical wavelength was set at 254 nm. Data acquisition and reduction were performed on a Hewlett Packard Model HP-3395 integrator (Palo Alto, CA, U.S.A.) Separations were accomplished on a

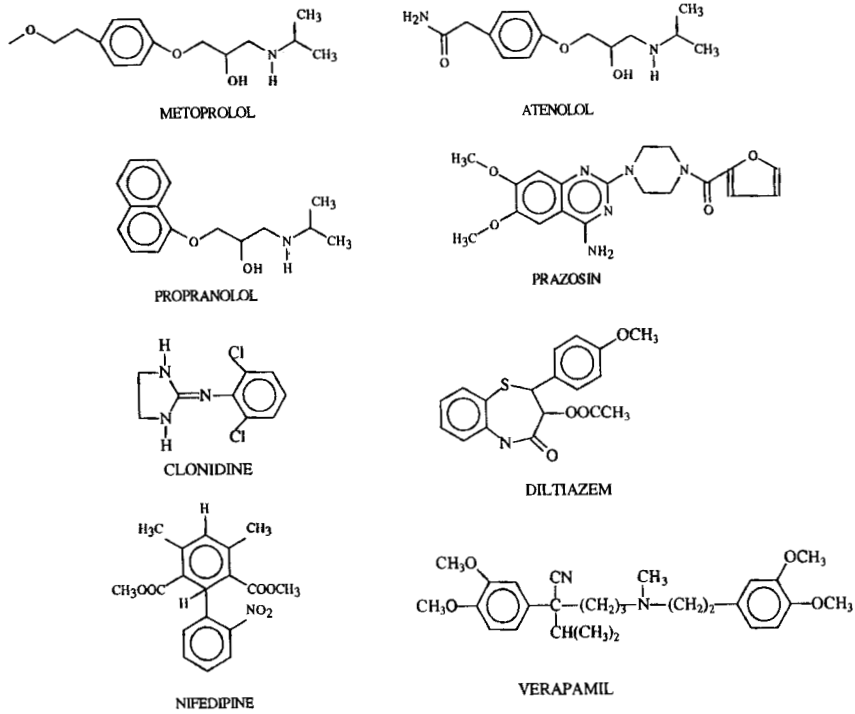

Figure 1 Chemical Structures of Selected Cardiovascular Agents

3-μm silica column (25 cm x 4.6 mm i.d., Phenomenex, Torrance, CA U.S.A.) The column was maintained at ambient temperature (23 ± 1°C).

Preparation of Mobile Phases:

Mobile phases containing aqueous phosphate buffers (pH 3, 4.5 and 6.0) and ionic strengths (3.125, 6.25 and 12.5 mM) combined with acetonitrile were prepared. The pH was adjusted with either 10%

phosphoric acid or 1N NaOH. All mobile phases were filtered through a 0.45 μm nylon-66 filter (MSI, Westborough, MA, U.S.A.) and degassed by sonication. The flow-rate was set at 1.0 mL/min.

Preparation of Stock Solutions

Individual stock solutions of propranolol, atenolol, and metoprolol at 25 μg/mL and verapamil, diltiazem, clonidine and prazosin at 50 μg/mL were prepared in aqueous acetonitrile mixtures containing the same proportion of acetonitrile as found in each mobile phase studied. Mixtures of the various stock solutions were prepared by using equal volumes of individual solutions. Triplicate injections were made into the liquid chromatograph.

RESULTS AND DISCUSSION

The purpose of this study was to demonstrate the applicability of using an underivatized silica stationary phase and organic-aqueous buffered eluents for the separation of cardiovascular agents of varied chemical structure. The model compounds were selected from a list of β-adrenergic blockers, calcium channel blockers, and α- adrenergic blockers. These drugs exhibit enough variation in chemical structure and functional group chemistry to provide a representative sample of cardiovascular compounds of pharmaceutical interest (see Fig. 1). Several mobile phases differing in organic modifier concentration, ionic

strength, and buffer pH were prepared and tested for the chromatographic separation of these analytes.

Aqueous 6.25 mM phosphate buffer pH 3.0 was mixed with varying acetonitrile concentrations to observe the effect of mobile phase composition on retention and column efficiency as measured by plate count (see Table 1). The mobile phase of 60:40 v/v phosphate buffer-acetonitrile gave the best overall separation within each class of cardiovascular agents. A decrease in the capacity factor (k') for the β-adrenergic blockers resulted from an increase in buffer concentration. This may be explained by other interactive forces, besides hydrogn bonding, which are more important for binding these drugs to the silica surface. These interactions may be dependent on nonpolar van der Waals

TABLE 1

Effect of Buffer and Organic Modifier Concentrations on Retention and Column Efficiency of Selected Cardiovascular Agents.

Analyte	40:60 v/v Buffer/CH_3CN k'	N[a]	50:50 v/v Buffer/CH_3CN k'	N	60:40 v/v Buffer/CH_3CN k'	N
Propranolol	2.27	6670	2.23	7100	2.28	8717
Atenolol	2.75	8776	2.33	7421	2.02	7358
Metoprolol	2.63	8212	2.50	8084	2.48	7916
Verapamil	2.61	8254	2.91	9703	3.72	14923
Diltiazem	2.73	8643	2.80	9154	3.16	11494
Nifedipine	0.39	1498	0.41	1948	0.48	5852
Clonidine	2.71	34575	2.59	8169	2.49	9902
Prazosin	3.01	40879	3.02	10148	3.28	12030

[a] Calculated as $N = 5.54\ (tr/W0.5)^2$

forces between the analyte, the acetonitrile and silanol or siloxane groups (14).

As buffer concentration was increased, k' for the calcium channel blockers also increased (see Table 1). This indicates a dependence on hydrogen bonding between the silica surface and a calcium channel blocker. With increasing concentrations of water in the mobile phase, hydrogen bonding is more extensive and the retention time of the analyte was shown to increase (15). The more extensive hydrogen bonding lattice may also stabilize ion pairing which may occur between the basic analyte and surface silanols.

Each of the α-blockers showed a different type of behavior with an increase in buffer concentration (see Table 1). Clonidine showed a gradual decrease in k' with increases in buffer concentration, while the k' of prazosin increased with an increase in buffer concentration. This indicated that prazosin is dependent on the hydrogen bonding strengthened by higher concentrations of water, while clonidine is dependent on major retention forces other than hydrogen bonding to silanol groups.

The cardiovascular agents studied displayed a general trend of increase in column efficiency with an increase in the buffer concentration of the mobile phase. Exceptions to this were atenolol and metoprolol, which gave decreases in column efficiencies with increased buffer

concentration. This behavior is explained by these drugs showing a decreased k' with about the same peak width.

The chromatographic separation of the selected cardiovascular agents was further investigated by changing the pH of the buffer in the mobile phase. The mobile phase was held constant at 60:40 v/v 6.25 mM phosphate buffer-acetonitrile with pH varied in the range 3-6.0. Table 2 shows the effect of increasing the pH of the buffer on the separation of each analyte. Those compounds possessing ionizable amino functional groups may undergo ion-pairing with an undefined number of strongly acidic silanol groups and these interactions are controlled directly by pH (14). The pKa of silanols at the silica surface is approximately 6.8 ± 0.2 (16). At lower pH, there are more unionized silanol groups which permit greater ability of the silica to hydrogen bond with an analyte. This increased hydrogen bonding network yields decreased analyte retention, thus showing hydrogen bonding to be a secondary interaction force to cause retention. Increases in pH yield higher ionization of silanol groups, which contribute to increased retention through a greater occurence of ion-pairing. All of the drugs chromatographed in this study showed an increase in retention with an increase in pH. This may show that hydrogen bonding and other aromatic interactions with siloxane groups are secondary interactions which affect selectivity, while ion pairing is the primary means of retention.

TABLE 2

Effects of Buffer pH on Retention and Column Efficiency of Selected Cardiovascular Agents.

Analyte	pH 3.0 k'	N*	pH 4.5 k'	N	pH 6.0 k'	N
Propranolol	2.28	8717	3.03	10125	7.54	9796
Atenolol	2.02	7358	2.75	8754	7.14	8820
Metoprolol	2.48	7916	6.97	11469	8.71	9481
Verapamil	3.72	14923	4.84	9449	15.07	9035
Diltiazem	3.16	11494	4.18	16516	11.10	12584
Nifedipine	0.48	5852	0.51	5708	0.69	6070
Clonidine	2.49	9902	3.36	11697	8.49	12473
Prazosin	3.28	12030	4.26	17250	6.23	16805

* Calculated as $N = 5.54 (tr/W0.5)^2$

There was a general trend of increased column efficiency with increased pH (see Table 2). Two exceptions which did not follow this trend were metoprolol and verapamil. These drugs appeared to form stronger ion-pair interactions with silanol groups as the pH increased. This may be seen by a multifold increase in k' with increases in pH. Metoprolol and verapamil were affected more by band broadening even though the capacity factors are high. The stronger ion-pair interaction of these two drugs provided an explanation for the increased band width. Diltiazem gave a slight increase in column efficiency with an increase in buffer pH from 3.0 to 4.5, but a decline in column efficiency between pH 4.5 to 6.0. This behavior is not readily explained.

The use of different ionic strengths of the mobile phase was also investigated. In these studies, the mobile phase composition was again

TABLE 3

Effect of Changes in Ionic Strength of the Buffer on Retention and Column Efficiency of Selected Cardiovascular Agents.

Analyte	3.125 mM k'	3.125 mM N*	6.25 mM k'	6.25 mM N	12.5 mM k'	12.5 mM N
Propranolol	4.39	9719	2.28	8717	1.30	6102
Atenolol	4.01	11607	2.02	7358	1.16	13739
Metoprolol	4.76	8155	2.48	7916	1.40	5267
Verapamil	6.75	11858	3.72	14923	2.10	10940
Diltiazem	5.89	11910	3.16	11494	1.78	15906
Nifedipine	0.73	18959	0.48	5852	0.49	10184
Clonidine	4.79	13143	2.49	9902	1.43	5362
Prazosin	6.09	12623	3.28	12030	1.87	7448

* Calculated as $N = 5.54\ (tr/W0.5)^2$

set at 60:40 v/v aqueous phosphate buffer pH 3-acetonitrile. The molarity of the buffer was varied from 3.125 to 12.5 mM to observe the effect of ionic strength on analyte chromatographic parameters. Table 3 shows the effect of increasing the ionic strength of the buffer on the separation of the three classes of drugs. At pH 3.0, sodium ions in the mobile phase may compete with the positively charged analyte for ionized silanols; therefore, if more sodium ions are added, the analyte will be displaced from the silica surface thus decreasing its retention (17). In this study, all of the drugs analyzed in each class showed a decrease in retention with an increase in ionic strength of the mobile phase.

According to the data, there was no general trend for retention of these cardiovascular agents with respect to column efficiency vs ionic

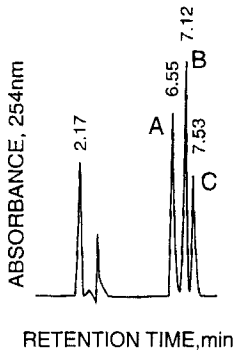

Figure 2 Typical HPLC Separation of Atenolol (A), Propranolol (B), and Metoprolol (C) on Underivatized Silica with 60:40 v/v pH 3 Phosphate Buffer - Acetonitrile at 1.0 mL/min

strength. For example, diltiazem showed a decrease in column efficiency from 3.125 - 6.25 mM, but an increase in column efficiency from 6.25 mM to 12.5 mM. This behavior is not easily explained by current knowledge of interactions of analytes with the silica surface.

The substitution of methanol as organic modifier in the mobile phase caused increased retention of the drugs as compared to equal amounts of acetonitrile. Diltiazem, verapamil, and prazosin showed the largest increases in the k' with methanol-containing mobile phases. This increased retention was accompanied by a decrease column efficiency as measured by N. Increased k' values for the various analytes may be attributed to the fact that methanol can participate in the hydrogen bonding lattice which retains analytes on silica gel. Acetonitrile is unable to form such bonds.

Figure 3 Typical HPLC Separation of Nifedipine (A), Diltiazem (B), and Verapamil (C) on Underivatized Silica with 60:40 v/v pH 3 Phosphate Buffer - Acetonitrile at 1.0 mL/min.

Typical HPLC separations of the selected cardiovascular agents using 60:40 v/v phosphate buffer pH 3.0-acetonitrile are shown in Figs. 2-4. Table 4 gives the analytical figures of merit for each drug in this HPLC system.

In summary, the HPLC separation of selected drug mixtures from three classes of cardiovascular agents were studied on underivatized

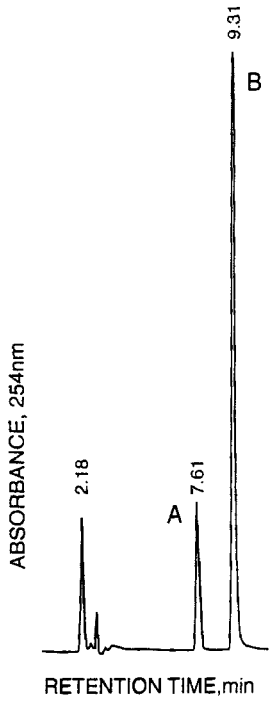

Figure 4 Typical HPLC Separation of Clonidine (A), and Prazosin (B) on Underivatized Silica with 60:40 v/v pH 3 Phosphate Buffer - Acetonitrile at 1.0 mL/min.

silica using aqueous acetonitrile mobile phases. The compounds were generally well-resolved with high column efficiencies. Although ion-pairing appears to be the primary interactive force between silanol groups and the analytes, other forces such as hydrogen bonding and hydrophobic interactions may also show effects on the chromatographic separation of these cardiovascular agents.

TABLE 4

Analytical Figures of Merit for Selected Cardiovascular Agents on Underivatized Silica with 60:40 v/v Phosphate Buffer pH 3.0-Acetonitrile.

Mix	Analyte	Rs	T_f[a]	N[b]	k'	Rt(min)	α
1.	Atenolol		1.19	7358	2.02	6.56	
		1.46					1.13
	Propranolol		1.00	8717	2.28	7.13	
		1.00					1.09
	Metoprolol		1.22	7916	2.48	7.54	
2.	Nifedipine		1.36	5852	0.48	3.25	
		13.93					6.58
	Diltiazem		0.95	11494	3.16	9.08	
		2.37					1.18
	Verapamil		1.33	14923	3.72	10.29	
3.	Clonidine		1.67	9902	2.49	7.61	
		3.11					1.32
	Prazosin		0.83	12030	3.28	9.32	

[a] Calculated at 5% peak height
[b] Calculated as $N = 5.54\ (tr/W0.5)^2$

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REFERENCES

1. Jane, I., "The Separation of a Wide Range of Drugs of Abuse by High-Pressure Liquid Chromatography", *J. Chromatogr.*, 111, 227, (1975).

2. Bidlingmeyer, B.A., Korpi, J., and Little, J.N., "Determination of Tricyclic Antidepressants Using Silica Gel with A Reversed-Phase Eluent", *Chromatographia*, 15, 83, (1982).

3. Lingeman, H., Van Muenster, H.A., Beymen, J.H., Underberg, W.J.M., and Hudshoff, A., "High-Performance Liquid Chromatographic Analysis of Basic compounds On Non-Modified Silica Gel and Aluminium Oxide with Aqueous Solvent Mixtures", *J. Chromatogr.*, 352, 261, (1986) .

4. Biffar, S.E., and Mazzo, D.J., "Reversed-Phase Determination of Famotidine, Potential Degradaters, and Preservatives In Pharmaceutical Formulation by High Performance Liquid Chromatography Using Silica as a Stationary Phase", *J.Chromatogr.*, 363, 243, (1986).

5. Law, B., "Analysis of Basic Compounds On a Silica Column with An Aqueous Methanol Eluent. The Use of Quantitative Structure-Retention Relationship In Metabolite Identification", *J. Chromatogr.*, 407, 1, (1987).

6. Schmidt, R.W. and Wolf, C. H.., "Use of Unmodified Silica with Buffered Aqueous Mobile Phase Mixtures for Selective Chromatography of Basic Drugs", *Chromatographia.*, 24, 713, (1987).

7. Gill, R. Osselton, M.D., and Smith, R.M., "International Collaborative Study of the Retention Reproducibility of Basic Drugs in High-Performance Liquid Chromatography on a Silica Column With A methanol-Ammonium Nitrate Eluent", *J. Pharm. Biomed. Anal.*, 7, 447, (1989).

8. Lampert, B.M. and Stewart, J.T., "Determination of Cocaine and Selected metabolites in Canine and Human Serum by Reversed Phase HPLC on Bare Silica., "*J. Chromatogr.*, 495, 153, (1989).

9. Lampert, B.M. and Stewart, J.T., "Determination of Non-Steroidal Anti-Inflammatory Analgesics in Solid Dosage Forms by Reversed Phase HPLC on Underivatized Silica", *J. Liq. Chrom.*, 504, 381, (1990).

10. Bidlingmeyer, B.A., Del Rios, J.K., and Korpi, J., "Separation of Organic Amine Compounds on Silica Gel with Reversed-Phase Eluents", *Anal. Chem.*, 54, 442 (1982).

11. Scott, R.P.W. and Kucera, P. "Solute-Solvent Interaction on the Surface of Silica Gel", *J. Chromatogr.* 149, 93, (1978).

12. Scott, R.P.W., and Kucera, P., "Solute-Solvent Interactions on The Surface of Silica Gel II", *J. Chromatogr.*, 171, 37, (1979).

13. Lampert, B.M. and Stewart, J.T., "Determination of Anabolic Steroids and Zeranol in Human Serum by Isocratic Reversed Phase HPLC on Silica", *J. Chromatogr.*, 12, 3231, (1990).

14. Cox, G.B., and Stout, R.W., "Study of The Retention mechanism For Basic Compounds on Silica Under "Pseudo-Reversed-Phase" Conditions", *J. Chromatogr.*, 323, 173, (1985).

15. Scott, R.P.W. and Traiman, S., "Solute-Solvent Interactions on The Surface of Silica Gel: III. Multilayer Adsorption of Water on The Surface of Silica Gel", *J.Chromatogr.*, 196, 193, (1990).

16. Iler, R.K., "The Chemistry of Silica", John Wiley and Sons, New York, NY, 1981.

17. Schmid, R.W., and Wolf, C. H., " Use of Unmodified Silica with Buffered Aqueous Mobile Phase Mixtures for Selective Chromatography of Basic Drugs", *Chromatographia*, 24, 713, (1987).

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