Reproducibility Study of Ion-Pair, High pH and Polar Reverse-Phase HPLC Methodologies

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Abstract

A common practice for analyzing poorly retained species on traditional alkyl stationary phases is to add an ion-pair reagent to the mobile phase. In this manner, highly polar analytes may be retained on traditional reversed-phase columns and their selectivity manipulated based on both ion-pair concentration and organic modifier percentage. The use of ion-pairing reagents, however, has disadvantages. In liquid chromatography/mass spectrometry (LC/MS) experiments, for example, the lack of volatility and ion-suppression effects of ion-pairing reagents limits their utility. In addition, the added complexity of mobile phase preparation often leads to poor reproducibility compared to simpler solutions.

Abstract (cont'd.)

Recent developments in HPLC stationary phase design have provided alternative approaches for the polar analyte retention dilemma. In one case, the use of high pH mobile phases on zirconia-based packings promotes greater hydrophobic retention for basic compounds. As another alternative, many polar reversed-phase chemistries provide retention of basic compounds at high organic modifier percentages without the need for the addition of ion-pairing reagents.

Abstract (cont'd.)

In this study, the reproducibility of methods developed using ion-pair reagents, high pH on zirconia-based columns, and normal-phase retention on polar reversed-phase stationary phases are compared. A separation based on a compendial ion-pair method selected from the USP is redeveloped using both high pH on a modified-zirconia column and at high organic percentage on a polar reversed-phase packing. For each system both repeatability and intermediate precision as described by the USP are assessed. The results of the study along with discussions of its implications are presented.

Introduction

The traditional approach for the retention and resolution of highly polar analytes on a common C18 phase is to add an ion-pair reagent to the mobile phase. Recent developments in HPLC stationary phase design have presented alternative methods for this dilemma. Two new approaches to solve this problem are to use a polar reverse-phase column exhibiting alternative retention mechanisms or high pH to neutralize basic analytes on a phase with extended pH stability. The goal of this study was to measure the reproducibility of each approach in terms of intra-column, inter-column and inter-laboratory precision.

Experimental

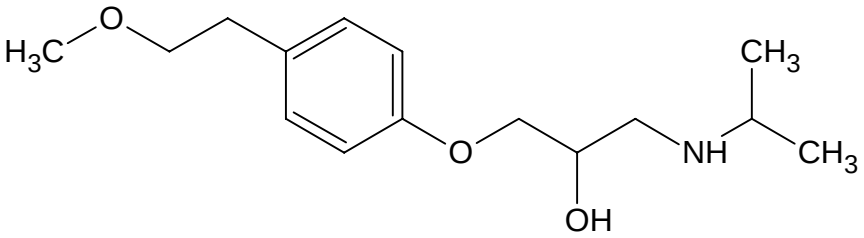
Test the three methods with a stationary phase appropriate to that method on three different columns, making multiple injections by two different analysts.

Table 1. Experimental Design

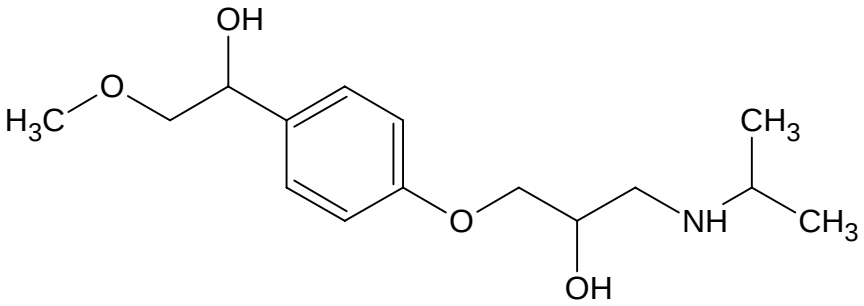
		Analyst #1		Analyst #2	
Method	**Stationary Phase - Support**	**Columns**	**Injections**	**Columns**	**Injections**
Ion-Pair	C18 - Silica	3	6 per column	1	6 per column
High pH	Polybutadiene-coated - Zirconia	3	6 per column	1	6 per column
Reverse-Phase	Pentafluorophenyl - Silica	3	6 per column	1	6 per column

Analytes

Figure A. Structures of Analytes



Metoprolol (MP)



Hydroxymetoprolol (HM)

Conditions

Ion-Pair Method

Column:	**Discovery C18, 25cm x 4.6mm ID, 5µm particles**
Mobile Phase:	USP Method, see below
Flow Rate:	1mL/min
Temp.:	35°C
Det.:	UV, 254nm
Inj.:	10µL
Sample:	200µg/mL each in mobile phase

USP 26, Metoprolol Tartrate Injection, pg. 1222:

Prepare a degassed solution by dissolving 961mg of 1-pentanesulfonic acid sodium salt (monohydrate) and 82mg of anhydrous sodium acetate in a mixture of 550mL of methanol and 470mL of water and adding 0.57mL of glacial acetic acid.

Conditions (cont'd.)

High pH Method

Column:	**Discovery Zr-PBD, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase:	80:20 water (10mM K_2PO_4, pH 12.0 with 1N NaOH):CH_3CN
Flow Rate:	1mL/min
Temp.:	35°C
Det.:	UV, 254nm
Inj.:	10µL
Sample:	200µg/mL each in mobile phase

Reverse-Phase Method

Column:	**Discovery HS F5, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase:	5mM ammonium acetate in 10:90 water:CH_3CN
Flow Rate:	1mL/min
Temp.:	35°C
Det.:	UV, 254nm
Inj.:	10µL
Sample:	200µg/mL each in mobile phase

Results
Intra-Column Precision

Metoprolol (MP) and Hydroxymetoprolol (HM)

Table 2. Intra-Column Precision, Ion-Pair Method

	Column #	Injection #	HM k'	MP k'	HM Area	MP Area
	1	1	0.182	0.616	54793	43575
	1	2	0.181	0.615	54720	43645
	1	3	0.182	0.615	54666	43332
	1	4	0.181	0.614	54607	43333
	1	5	0.181	0.614	54712	43451
	1	6	0.181	0.614	54945	43493
average			0.181	0.615	54741	43472
standard dev			0.001	0.001	118	127
%CV			**0.285**	**0.133**	**0.215**	**0.292**

Inter-Column Precision

Table 3. Inter-Column Precision, Ion-Pair Method

	Column #	HM k'	MP k'	HM Area	MP Area
average	1	0.181	0.615	54741	43472
standard dev	1	0.001	0.001	118	127
%CV	1	0.285	0.133	0.215	0.292
average	2	0.179	0.608	55481	43928
standard dev	2	0.001	0.001	264	146
%CV	2	0.288	0.085	0.477	0.332
average	3	0.190	0.631	54999	43580
standard dev	3	0.001	0.001	104	125
%CV	3	0.272	0.100	0.188	0.288
average	1, 2, 3	0.185	0.619	55240	43754
standard dev	1, 2, 3	0.007	0.016	340	246
%CV	1, 2, 3	**3.960**	**2.664**	**0.616**	**0.562**

Inter-Laboratory Precision

Table 4. Inter-Laboratory Precision, Ion-Pair Method

	Column #	Analyst	HM k'	MP k'	HM Area	MP Area	Normalized Area Ratio (HM/MP)
average	1	A	0.181	0.615	54741	43472	1.259
standard dev	1	A	0.001	0.001	118	127	
%CV	1	A	0.285	0.133	0.215	0.292	
average	1	B	0.181	0.607	149739	113160	1.323
standard dev	1	B	0.001	0.001	487	164	
%CV	1	B	0.313	0.169	0.326	0.145	
average	1	A, B	0.181	0.611	102240	78316	1.291
standard dev	1	A, B	0.000	0.006	67174	49277	0.045
%CV	1	A, B	**0.121**	**0.936**	**65.70**	**62.92**	**3.506**

Summary of Data

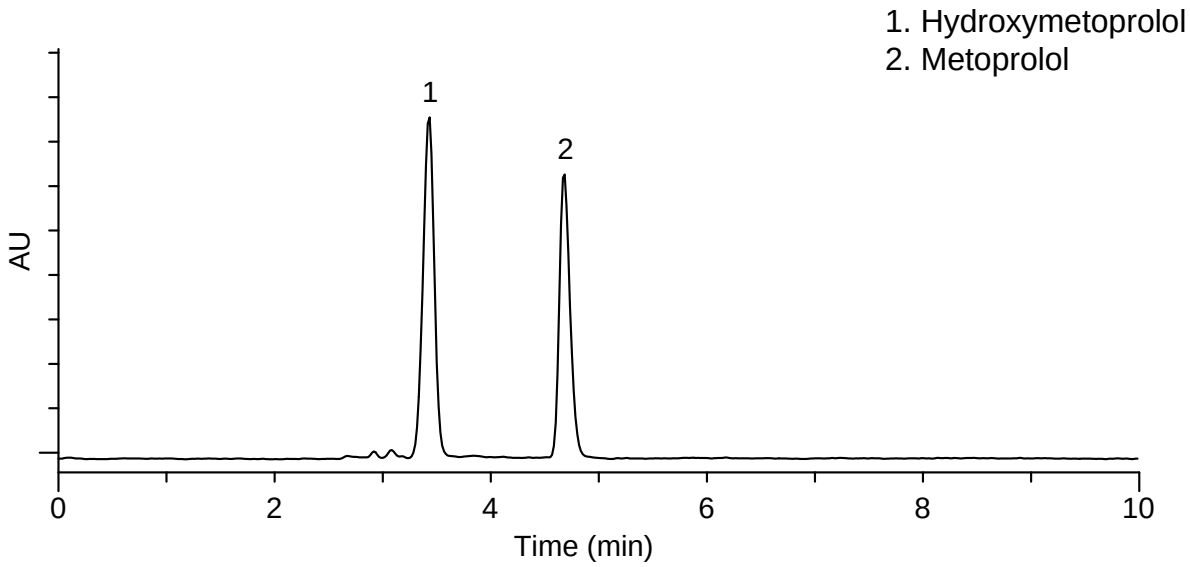
Table 5. Summary of Data, All Methods

	%CV of Metoprolol (MP) k'		
	Intra-Column	**Inter-Column**	**Inter-Laboratory**
Ion-Pair	0.133	1.938	0.936
High pH	0.210	1.784	1.234
Polar Reverse-Phase	0.878	4.090	4.211
	%CV of Metoprolol (MP) Area		
	Intra-Column	**Inter-Column**	**Inter-Laboratory**
Ion-Pair	0.292	0.546	-
High pH	1.171	0.355	-
Polar Reverse-Phase	3.009	0.927	-

Representative Chromatogram

Ion-Pair Method

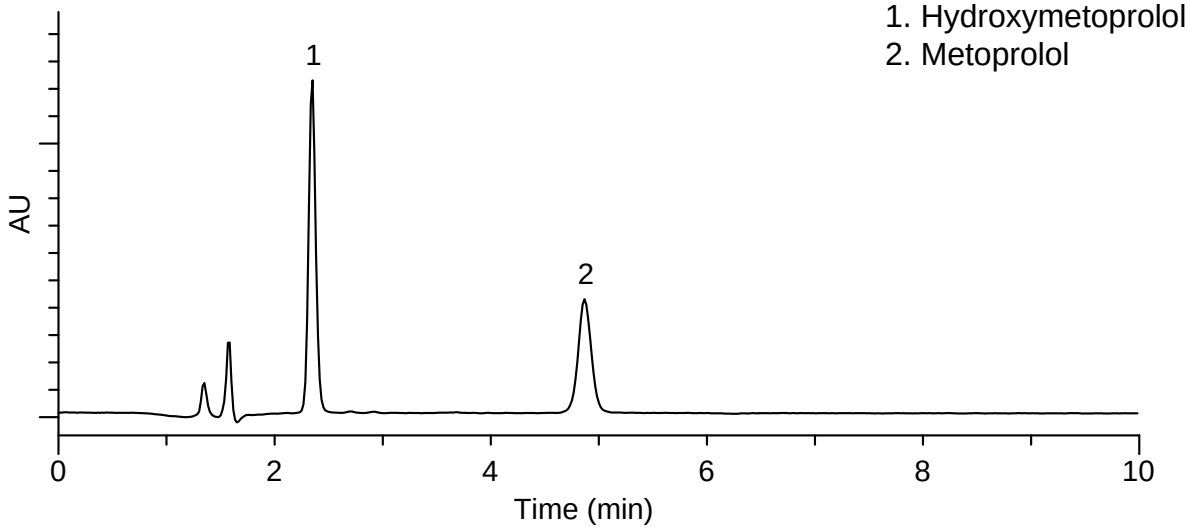
Figure B. Representative Chromatogram, Ion-Pair Method



Representative Chromatogram

High pH Method

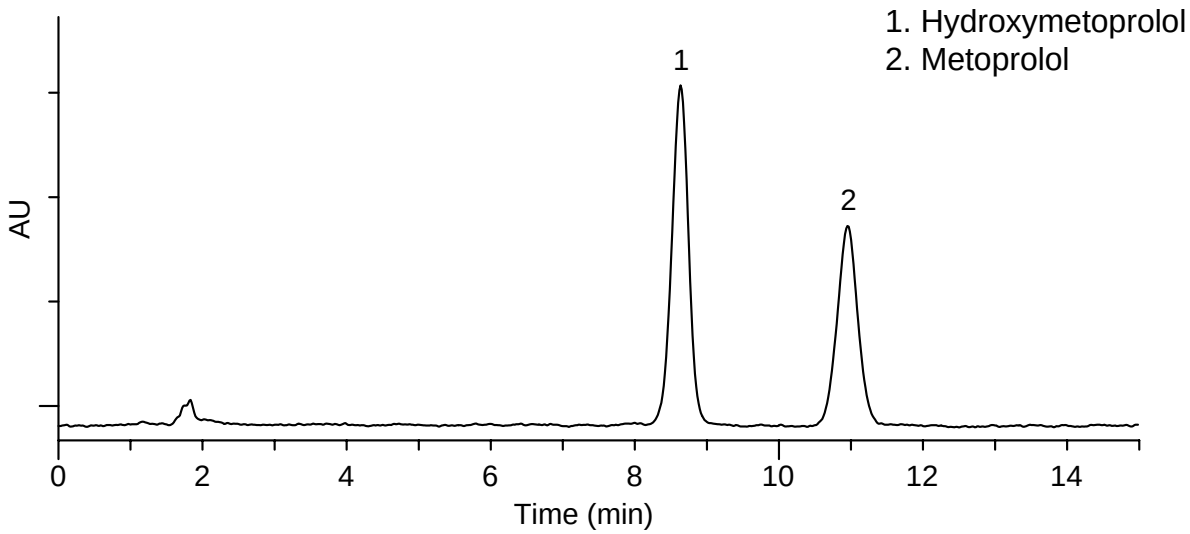
Figure C. Representative Chromatogram, High pH Method



Representative Chromatogram

Reverse-Phase Method

Figure D. Representative Chromatogram, Reverse-Phase Method



Conclusions

All techniques provide acceptable precision

Each approach has its own advantages and disadvantages

Ion-Pair Method

Advantages

- **utilized common C18 phase**
- **generally appropriate for UV detection**

Disadvantages

- **complex mobile phase preparation**
- **gradients not recommended**
- **incompatible with LC/MS**
- **long equilibration times**

Conclusions (cont'd)

High pH Method

Advantages

- primarily simple "hydrophobic" interaction
- predictable behavior based on log P

Disadvantages

- not optimum for LC/MS
- mobile phase outside "comfort zone" for many chromatographers

Reverse-Phase Method

Advantages

- highly LC/MS compatible
- simple mobile phase preparation

Disadvantages

- complex retention mechanisms – difficult to predict
- slight increase in variability