MANIPULATING CHROMATOGRAPHIC SELECTIVITY USING AUTOMATED CONTROL OF MOBILE PHASE PH

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INTRODUCTION

Chromatographic resolution is best maximized by manipulations that change the selectivity of the separation conditions. For reversed-phase methods, numerous manipulations can affect selectivity, including for example, strong solvent, bonded phase, temperature, as well as other parameters. While mobile phase pH is recognized as a powerful tool for altering selectivity, particularly for ionizable analytes, this parameter is seldom used in separation development protocols since the preparation of buffered mobile phases is time consuming and labor-intensive. In common practice, only widely separated pH conditions are used to maximize retention. For groups of related compounds, however, small changes in pH can be more effective because the analytes behave as though partially charged. We describe here the automated screening of this separation variable in a software controlled system. A quarternary solvent pump is used with specific algorithms to permit programming directly in units of pH and buffer concentration. Buffered mobile phases are prepared on demand from concentrated stocks using the solvent proportioning capabilities of the UPLC pump. A series of small incremental changes in pH can be evaluated to identify the point of maximum selectivity. As expected, the effective pH ranges center around the pK's of the analyte molecules. The approach is particularly useful for structurally related molecules that differ slightly in pK. In the present study, an alternative protocol using continuous gradients of pH has been tested. The software embodying the control algorithms can be used to generate gradients of pH. Buffer systems have been developed for the reversed phase application that give stable, predictable, and reproducible gradients over the required pH range while maintaining compatibility with MS detection.

AUTO•BLEND PLUS™

Auto•Blend Plus™ is a tool offered with the ACQUITY UPLC® H-Class Quaternary Solvent pump. It is a combination of instrument characteristics and software algorithms with a user interface that lets us work in the units of pH and solvent composition. Based on well-understood principles, Auto•Blend Plus™ calculates the proportion of solvents needed to deliver the user requested mobile phase composition. pH and solvent concentration can be changed independently, simultaneously and accurately.

Auto•Blend Plus™ calculates the proportion of solvents needed to deliver the requested pH based on a user defined buffer system. The buffer system includes the concentration of acid and base to be used along with organic and aqueous solvent strength. The calculation can either be based on the pKa or on an empirical table. In reversed phase separations the amount of organic has an influence on the pH. The empirical table provides a reliable basis for calibration.

METHODS

Instrumentation

QSM - ACQUITY UPLC H-Class Bio System
SM-FTN -ACQUITY UPLC Sample Manager Flow Through Needle
ISM - Isocratic Solvent Manager
QDa - ACQUITY QDa Mass Detector (used for reversed-phase)
PDA - ACQUITY Photodiode Array Detector

All from Waters, Milford, MA

Column

ACQUITY UPLC BEH C18, 130Å, 1.7 µm, 2.1 mm X 50 mm
XBridge BEH C18 Direct Connect HP Column,130Å,10µm,2.1 X 30 mm

Sample

Reversed Phase Test Mix:
(0.45 mg/mL Sulfadimethoxine, 0.45 mg/mL Terfenadine, 0.45 mg/mL Reserpine, 0.45 mg/mL Acetaminophen, 0.45 mg/mL Caffeine, 90ug/mL Acetamidophenol, 90ug/mL Acetanilde, 90ug/mL Acetylsalicylic Acid, 90ug/mL Phenacetin, 0.20 mg/mL Salicylic Acid, 0.20 mg/mL 3-benzoylpyridine, 0.20 mg/mL Cortisone, 0.20 mg/mL 4-nitroaniline,0.20 mg/mL 4,4'-biphenol

Conditions

Mobile Phase:

ISM:	50:50 Water:Methanol
QSM:	
Acid:	50mM Maleic Acid + 25mM Citric Acid +25mM Succinic Acid
Base:	50mM 4-methyl Morpholine + 50mM Morpholine +50mM Ammonium Hydroxide + 50mM Piperidine
Aqueous:	Water
Organic:	Acetonitrile

Conditions:

ISM:	0.3mL/min
QSM:	Isocratic at 10% Acetonitrile 0.6mL/min 40°C pH with Auto•Blend Plus as in Figure 1

RESULTS

%B	pH	%B	pH
0	2.22	11	6.99
1	2.32	12	7.78
2	2.45	13	8.41
3	2.63	14	8.89
4	2.88	15	9.32
5	3.26	16	9.85
6	3.79	17	10.4
7	4.38	18	10.9
8	4.97	19	11.3
9	5.57	20	11.5
10	6.14		

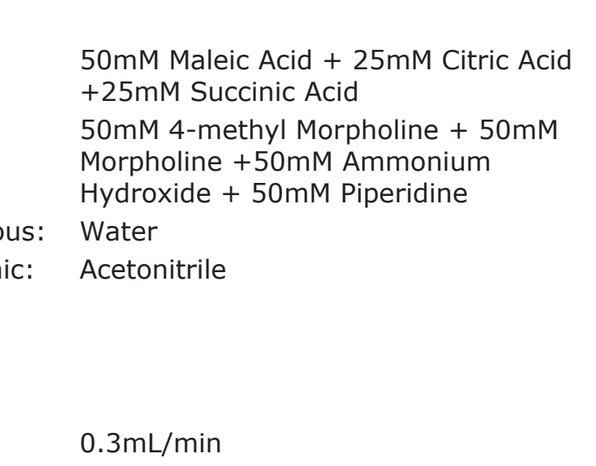

Figure 1. Relationship between Mobile Phase pH and proportion of Buffer B. Standard mixtures of the Acid and Base Components of the Multi-buffer System were pipetted and the pH measured with a meter. Each sample was 80% water. The composition is expressed as % B, and %A is 20% − %B. The empirical table derived from these 21 points is used by the software to calculate the percentages required to deliver the programmed pH.

RESULTS

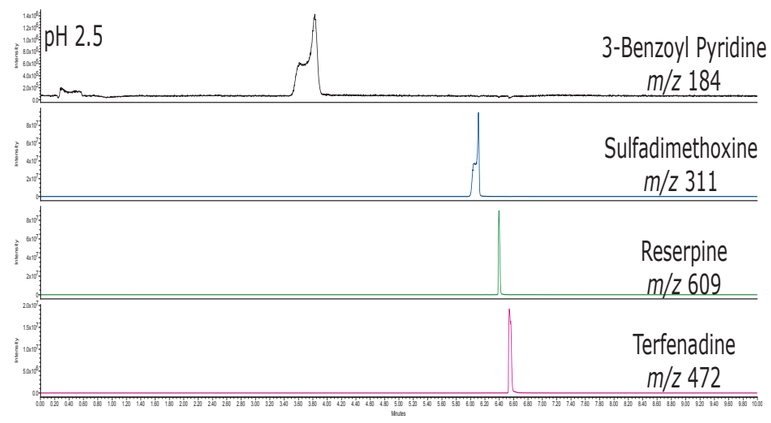

Figure 2. Mobile phase at pH 2.5. The mixture was separated in an isocratic method with 90% Water: 10% Acetonitrile at pH 2.5. Peak shape is good with injection artifact effects for 3-Benzoyl Pyridine and Sulfadimethoxine. The last three peaks are resolved, but are too closely spaced to consider reducing run time or analyzing extreme concentration ratios.

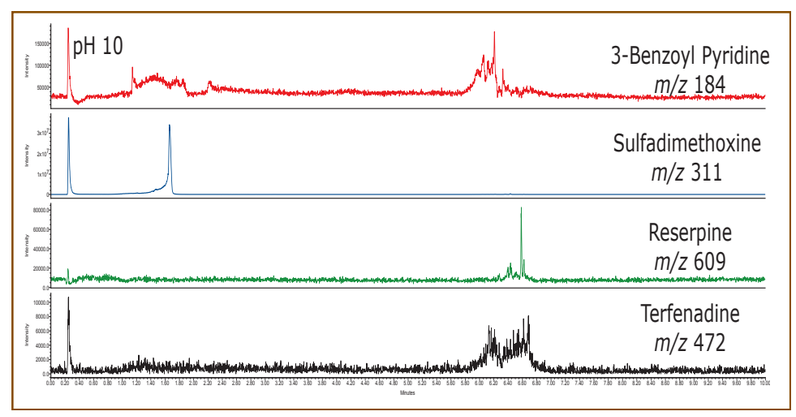

Figure 3. Mobile phase at pH 10.0. The mixture was separated in an isocratic method with 90% Water: 10% Acetonitrile at pH 10. The selectivity for 3-Benzoyl Pyridine and Sulfadimethoxine is reversed. The detector signal is, however, diminished with such loss of sensitivity that the conditions are not useful for such an analysis.

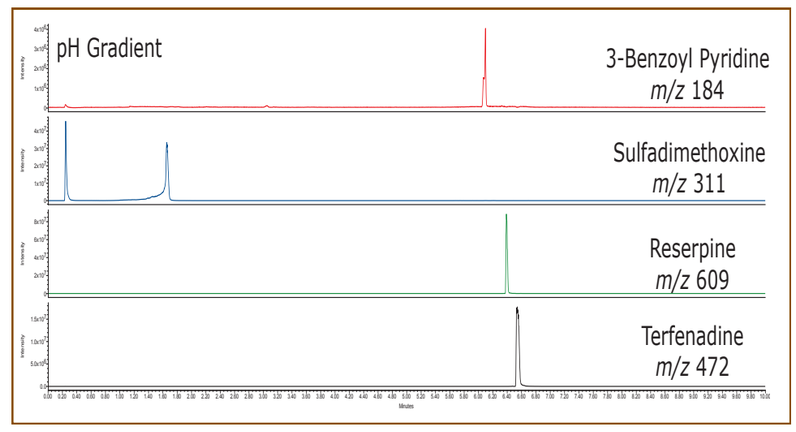

Figure 4. Mobile phase as pH Gradient, pH 10 to pH 2.5. The mixture was separated in an isocratic method with 90% Water: 10% Acetonitrile using a gradient of decreasing pH. The order of elution is similar to that observed at constant pH 10. The detector response is, however, much better than the pH 10 analysis. For this combination of sample and mobile phase, the availability of pH gradients provides and additional tool for manipulation of selectivity.

CONCLUSION

- **Mobile phase buffers giving stable pH control over a wide pH range can be generated from mixtures of weak acids and weak bases**
- **Mobile phase pH can be programmed using Auto•Blend Plus**
- **Chromatographic selectivity can be different in pH gradients as compared to constant pH elution**

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