Utilization of Liquid Chromatography at Elevated pH and Elevated Pressures in Quantitative Bioanalysis

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INTRODUCTION

Increasing the sensitivity of bioanalytical assays whereby potential new medicines and their metabolites are quantitated is often required during development of new therapeutics for disease. This is primarily due to the fact that many new medicines have become more potent enabling smaller doses to be administered thus reducing the circulating levels in patient biofluids. Further, the detection of metabolites associated with medicines is of particular importance related to the potential of toxicity that may be associated with their formation in humans.

Sensitivity gains can be accomplished for bioanalytical assays in a variety of ways including: the use of tandem quadrupole mass spectrometry, reduction in chromatographic column diameter and increased amount of sample injected onto the analytical system. Additionally, the introduction of chromatographic columns packed with sub 2 micron particles has shown the capability of increasing sensitivity in quantitative bioanalytical applications due to the inherent increased chromatographic efficacy offered by the technique.

The approach of mobile phase modification relative to pH is a simple but often overlooked approach to increased sensitivity. Increases in selectivity, improved peak shape and increased MS response are often the result of operating under high pH conditions. Here we present the utilization of chromatographic columns packed with sub 2 micron particles run with elevated pH mobile phases as a means to increase sensitivity lowering LLOQ in quantitative bioanalytical applications.

Figure 1. Waters ACQUITY UPLC® Coupled With Waters Xevo™ TQ MS.

METHODS

Compounds were spiked into rat plasma at a concentration of 10 ng/mL and precipitated with acetonitrile using a 2:1, acetonitrile : plasma ratio. The sample was then centrifuged at 13,000 RCF for 5 minutes. The supernatant was removed diluted 1:1 with water and injected onto the UPLC® /MS system.

LC conditions

LC System: Waters® ACQUITY UPLC® System
Column: ACQUITY UPLC BEH C18 Column 2.1 x 50 mm, 1.7 µm
Column Temp: 40 °C
Flow Rate: 600 µL/min.
Mobile Phase A: 0.1 % Ammonium Hydroxide (pH 10) 0.1 % Formic Acid (pH 2.5)
Mobile Phase B: MeOH or MeCN
Gradient: 5-95% B/2 min.

MS conditions

MS System: Waters Xevo™ TQ MS
Ionization Mode: ESI positive ion mode
Desolvation Temp: 600 °C
Desolvation Gas: 1000 L/Hr
Source Temp: 150 °C

Compound	Capillary (V)	Cone (V)	Collision Energy	Transition (m/z)
Alprazolam	1.0	25	20	309 > 281
Benzimidole	1.5	53	23	119 > 92
Cefazoline	1.5	18	10	455 > 323
Irbesartan	1.0	42	26	429 > 207
Fluticasone propionate	3.0	30	17	501 > 293
Nefazodone	1.0	40	27	470 > 274
Nefazoline	1.0	21	30	211 > 141
Nimodipine	1.0	15	10	419 > 343
Oxybutynin	1.0	60	37	358 > 72
Testosterone	3.0	27	16	289 > 97

RESULTS

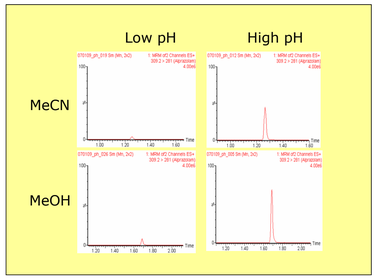


Figure 2: Response of Alprazolam by ESI positive MS detection under low pH (2.5), high pH (10.0) aqueous mobile phases combined with MeCN or MeOH organic mobile phases. A ten fold increase in MS response was obtained utilizing high pH mobile phases while maintaining good peak shape.

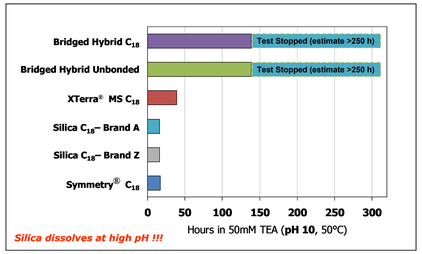


Figure 3: Lifetime study of columns packed with Bridged Hybrid particles (BEH) under high pH conditions. Traditional silica based particles are incapable of operating with high pH mobile phases.

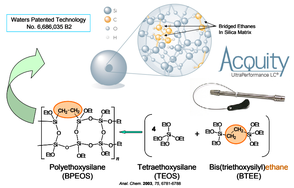
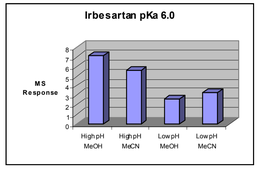
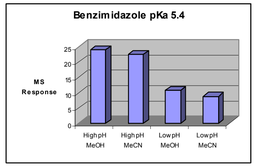
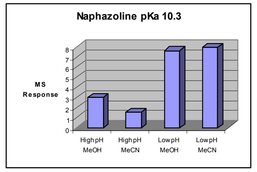
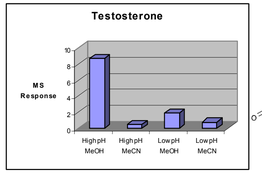


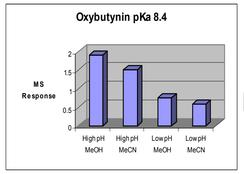
Figure 4: Bridged hybrid particle chemical composition.

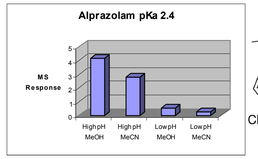


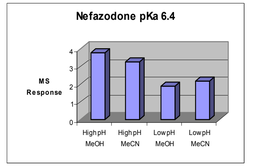


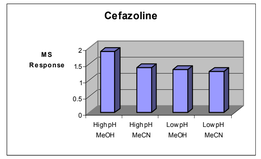


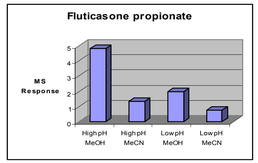












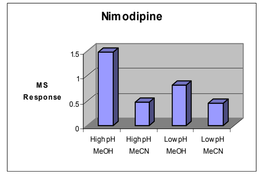


Figure 5: Response of various compounds by ESI positive MS detection under low pH (2.5), high pH (10.0) aqueous mobile phases combined with MeCN or MeOH organic mobile phases. MS response is chemical dependant.

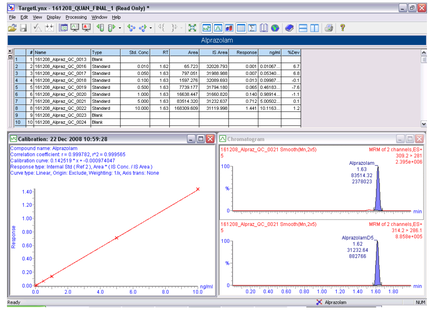


Figure 5: Unparalleled sensitivity: The combination of Waters UPLC® , high pH mobile phase and Xevo TQ MS gave a LLOQ of 10 pg/mL for Alprazolam in protein precipitated rat plasma.

CONCLUSION

- **Waters ACQUITY UPLC® BEH columns operate in a wide pH range.**
- **The utilization of high pH mobile phases has shown increases in MS response for a variety of compounds under positive electrospray ionization.**
- **Up to a 10 fold increase in MS response was obtained utilizing high pH mobile phases.**
- **The combination of Waters UPLC® , high pH mobile phase and Xevo™ TQ MS gave a LLOQ of 10 pg/mL for Alprazolam in protein precipitated rat plasma.**

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