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Application of High pH Separations for the Quantification of Small and Large Molecules

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Bioanalysis Studies and High pH Mobile Phases with ESI Positive

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- Mess, J., Lahaie, M., Furtado, M., Garofolo, F., 2009 *Bioanalysis,* 1(8): 1419-1430
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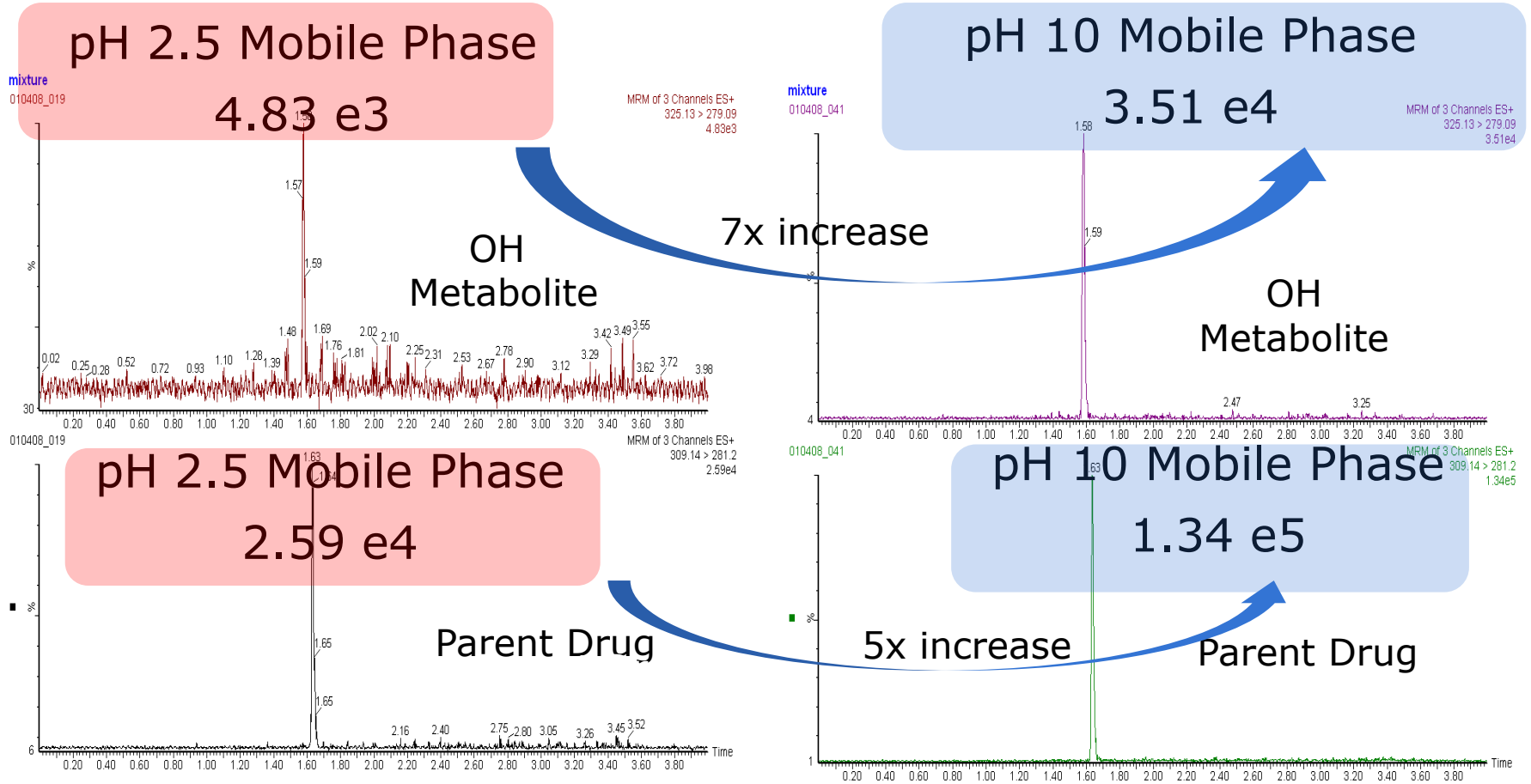
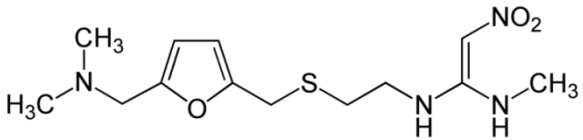
Increasing Ionization Efficiency
ESI Positive Mode

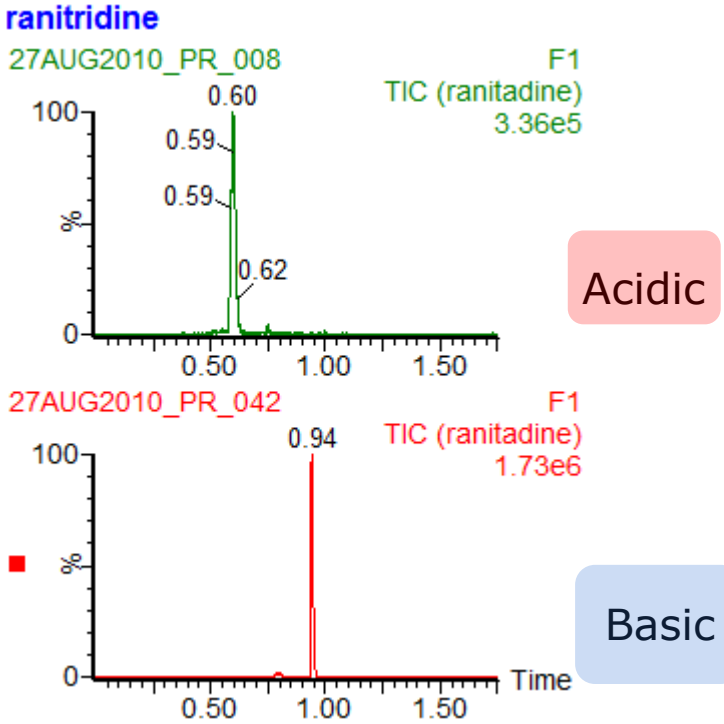


Mather,J., Rainville, P.D., Smith, N.W., Plumb, R.S. 2010 *Journal of Drug Testing and Analysis 2 (1) 11-18*

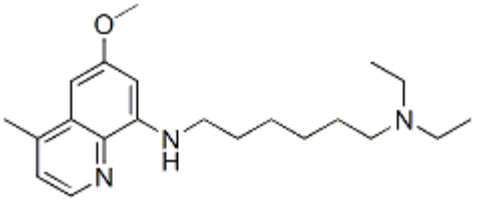
pH 10 Mobile Phases Chromatographic Affect

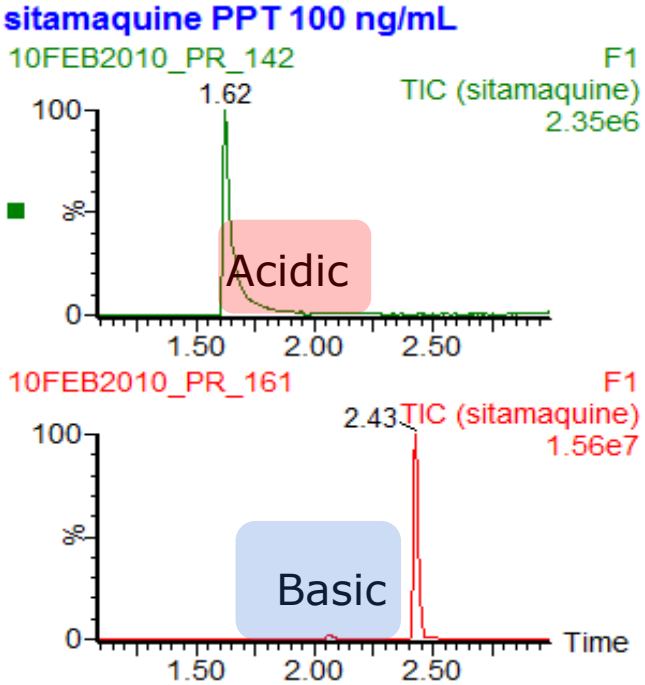
- ***Increasing RT of Polars***





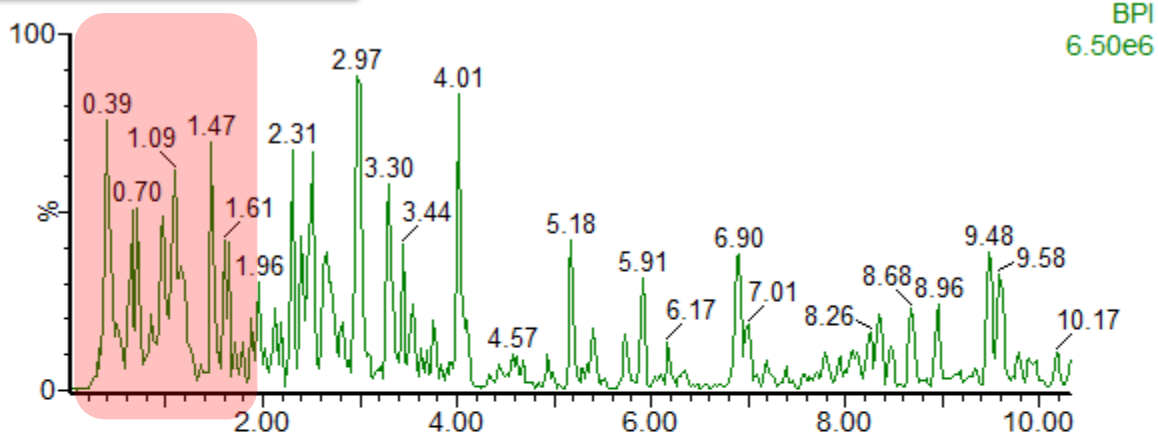
- ***Peak Shape Improvement***



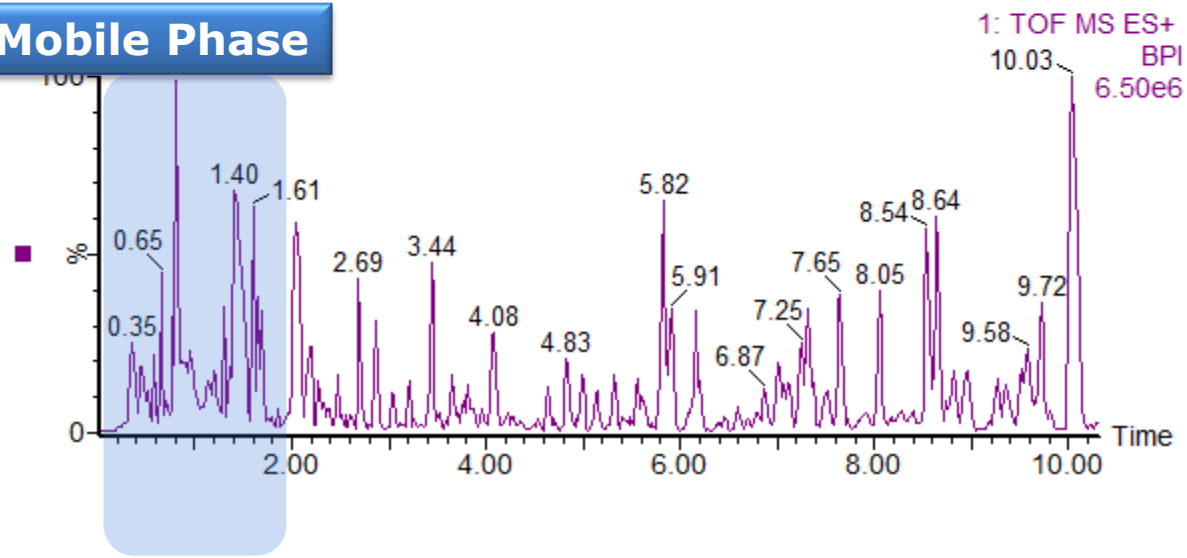


Human Urine Results

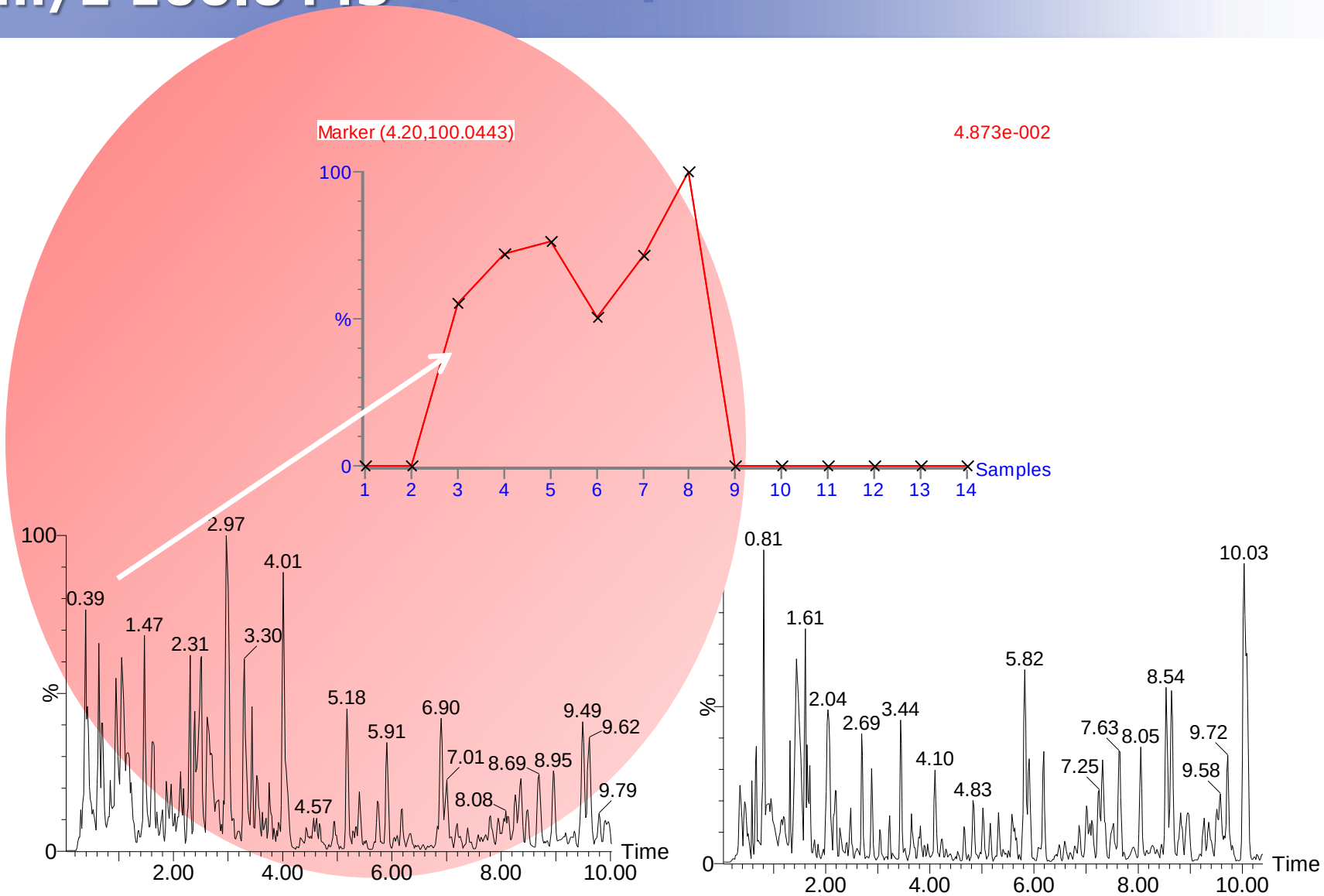
Acidic Mobile Phase



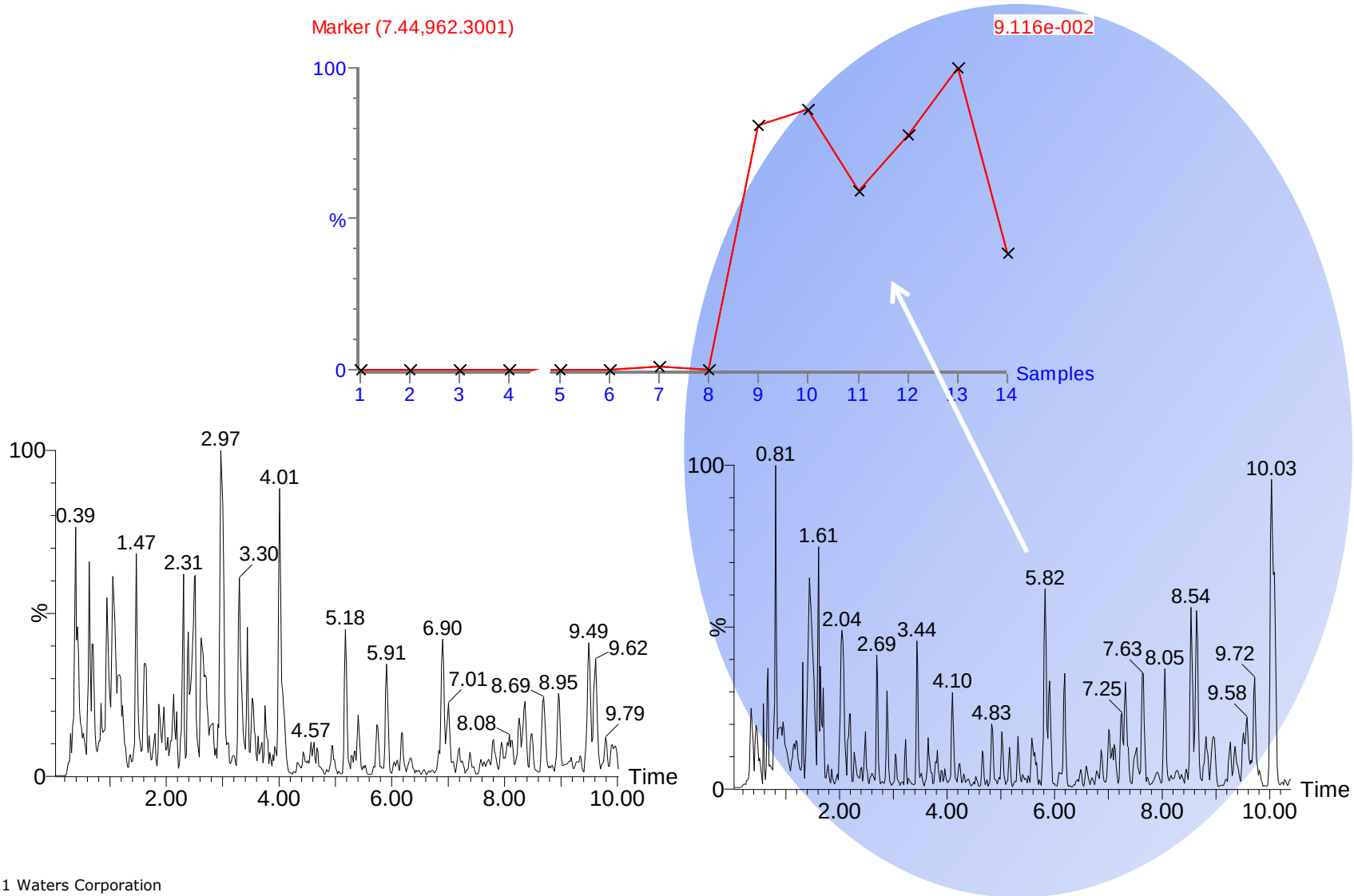
Basic Mobile Phase



m/z 100.0443



m/z 962.3001



Effect of Peak Response For Alprazolam at Low and High pH

Bioanalytical method for alprazolam using UPLC/MS/MS

Drug Testing and Analysis

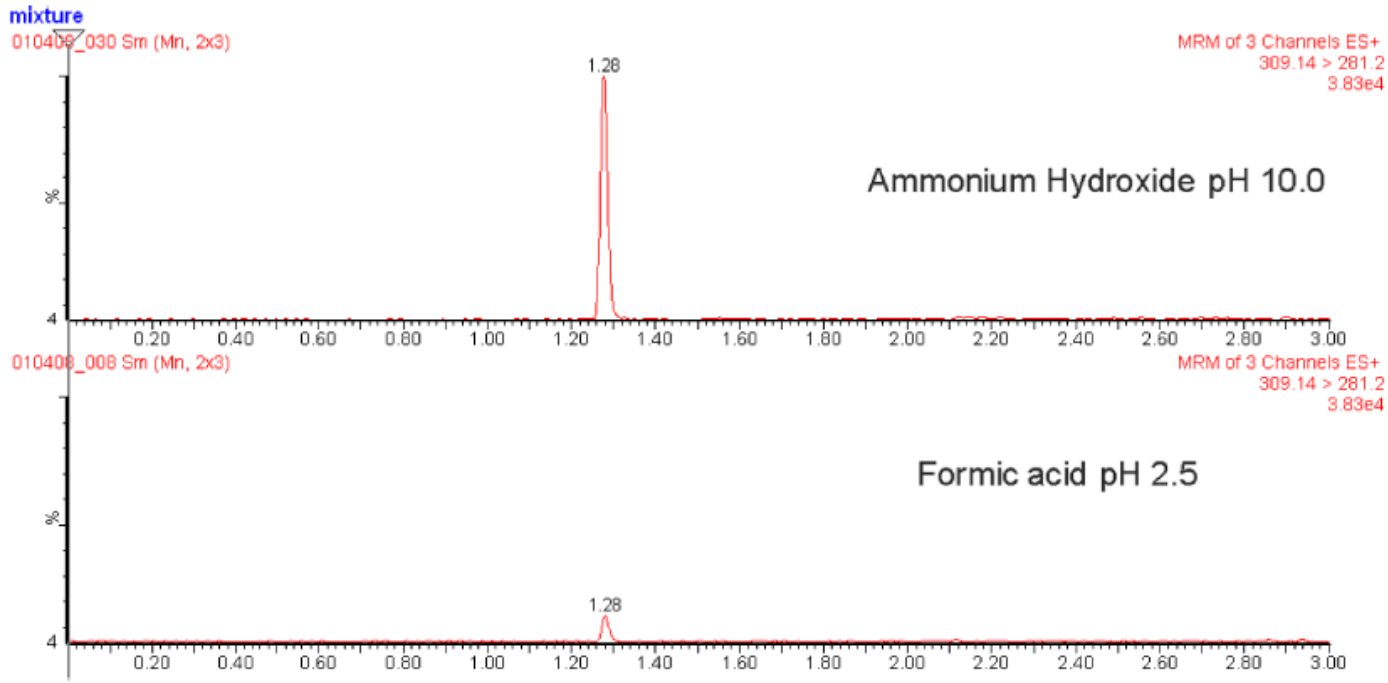


Figure 1. Comparison of MS peak response for alprazolam in basic (top) and acidic (bottom) mobile phases.

Effect of Organic Modifier on Peak Response

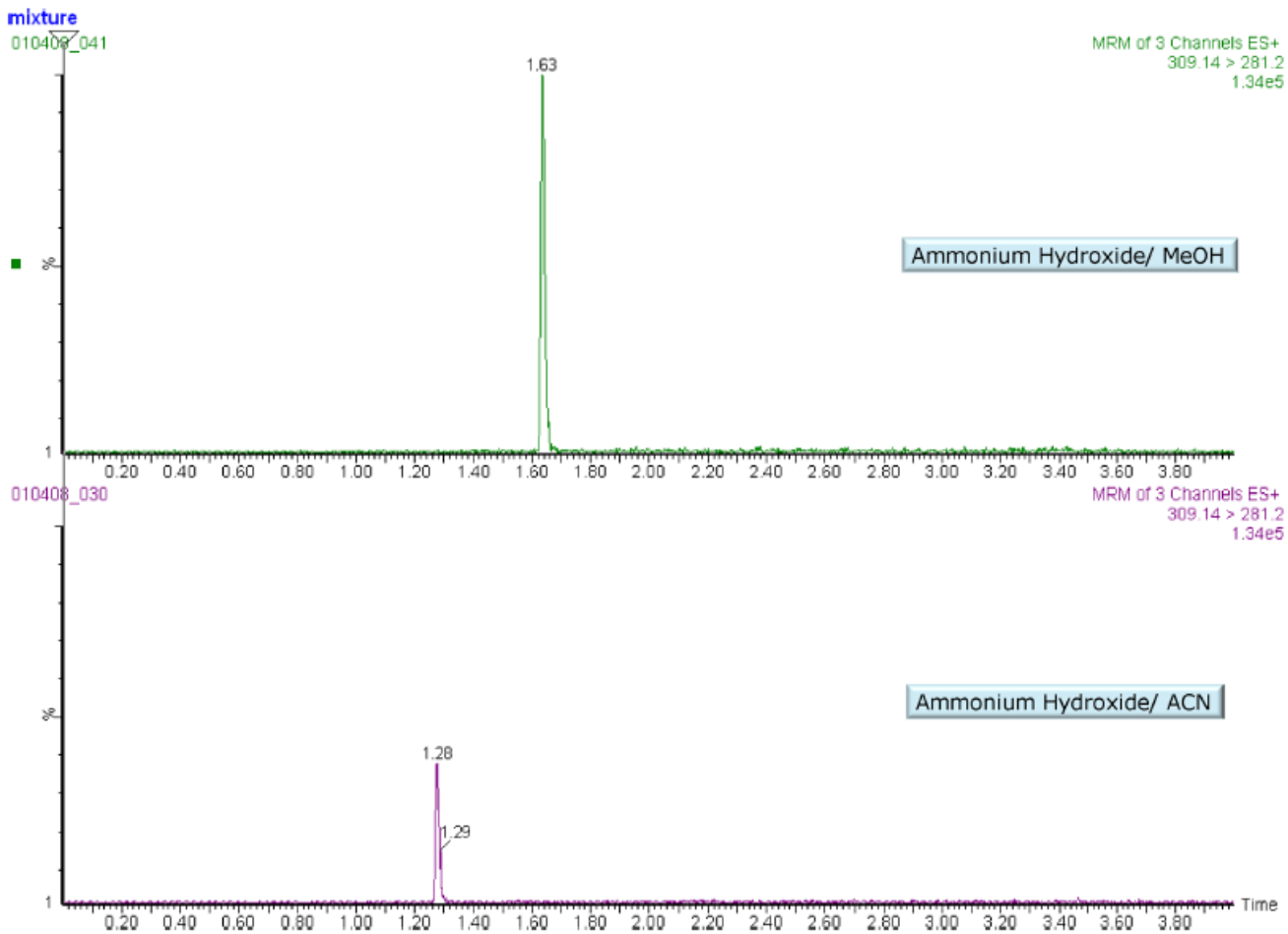


Figure 2. Comparison of MS peak response for alprazolam in basic - methanol (top) and basic - acetonitrile (bottom) mobile phases.

Bioanalytical Assay For Alprazolam With Protein Precipiated Plasma

Bioanalytical method for alprazolam using UPLC/MS/MS

Drug Testing and Analysis

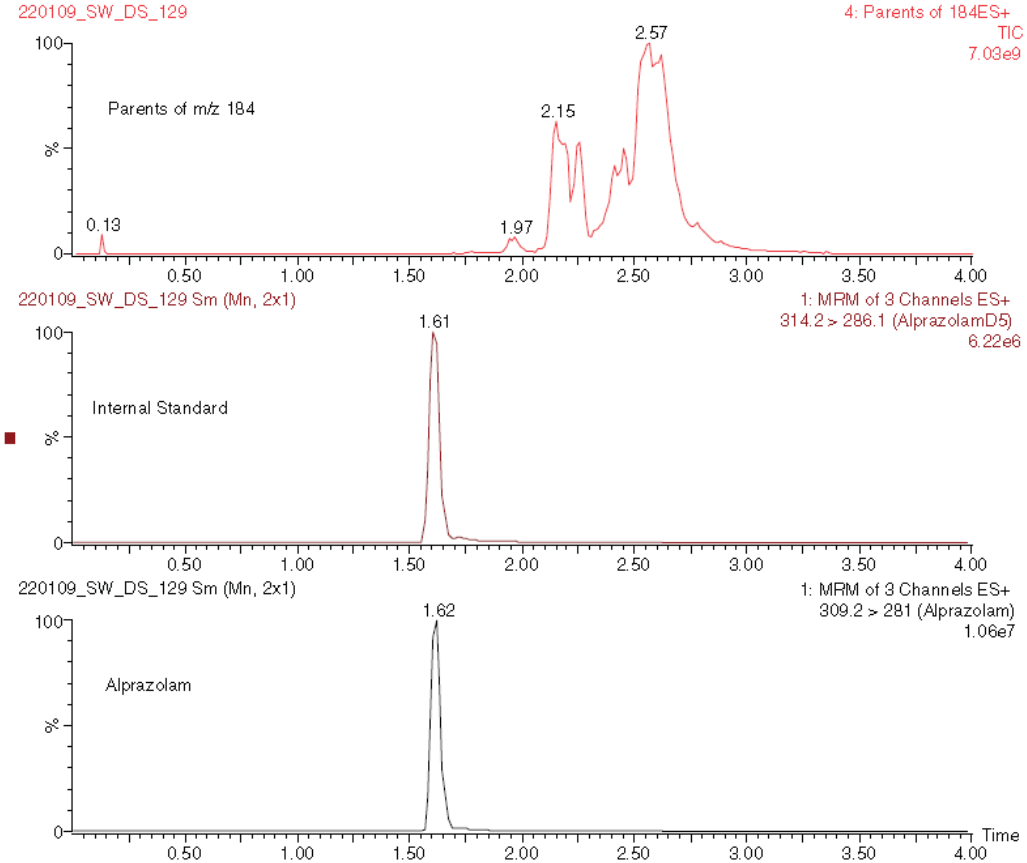


Figure 3. Positive ion LC/MS/MS chromatogram of alprazolam and D5-alprazolam internal standard in protein-precipitated plasma. Top chromatogram: precursor ion scan LC/MS chromatogram (precursor *m/z* 184); middle chromatogram: D5 internal standard MRM analysis (*m/z* 314 > 286); lower chromatogram: alprazolam MRM analysis (*m/z* 309 > 281).

Internal Standard and Metabolite

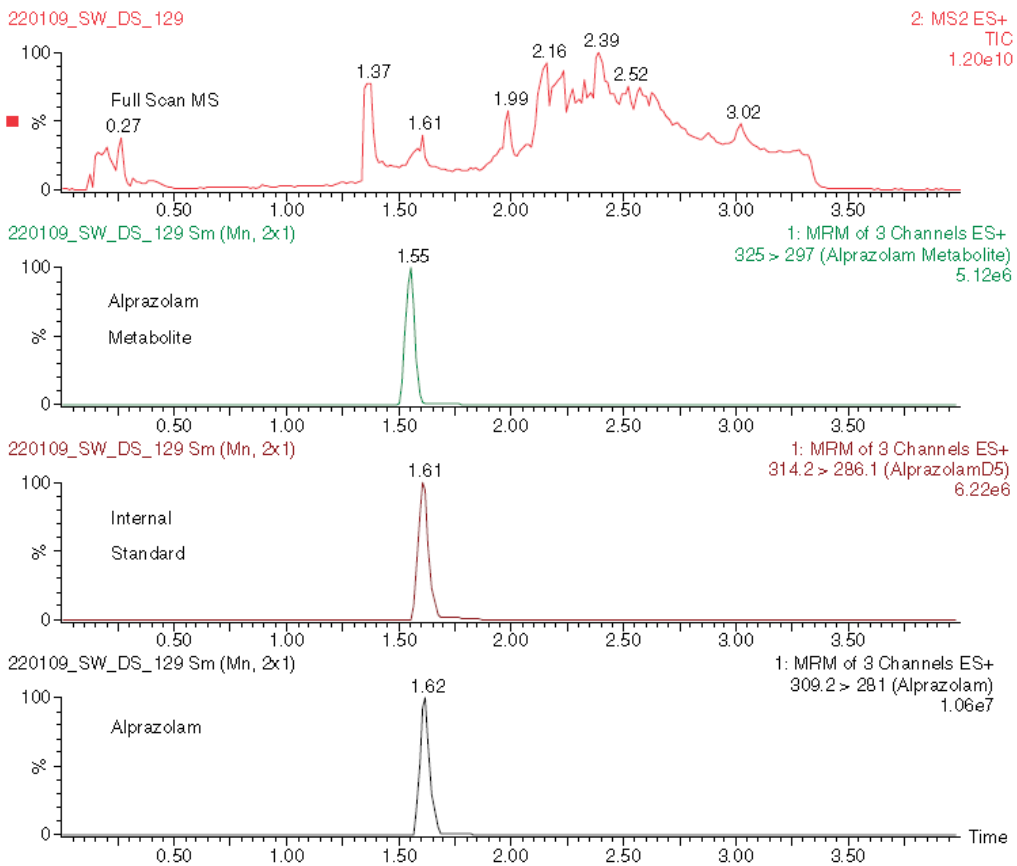
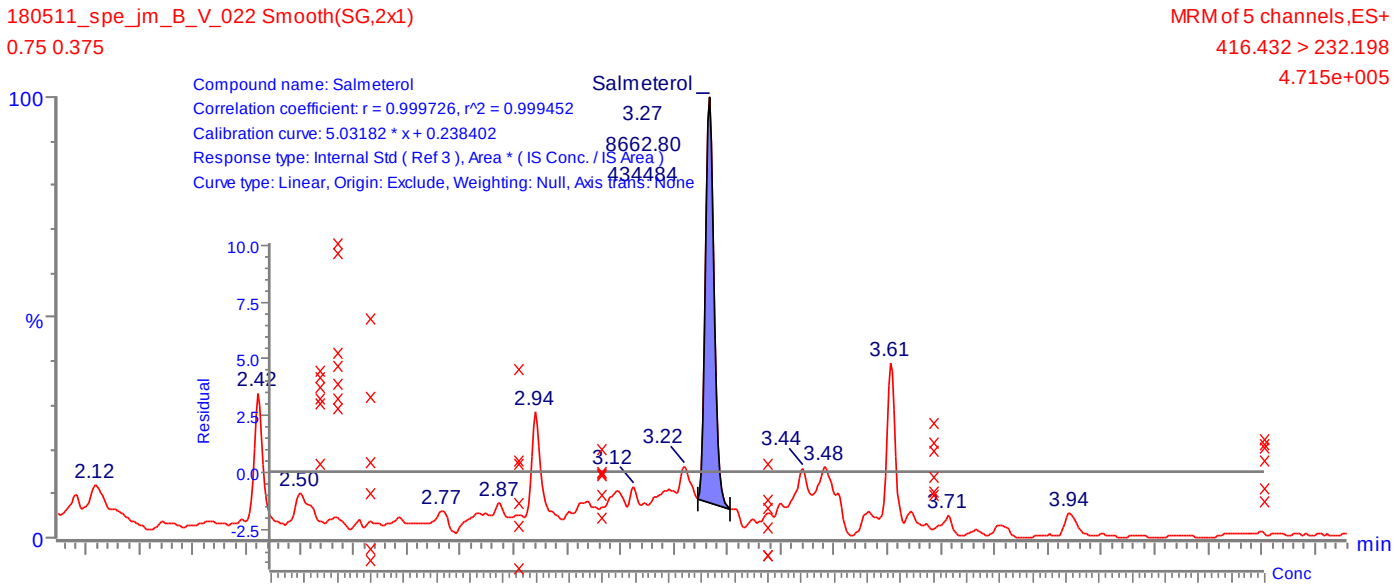
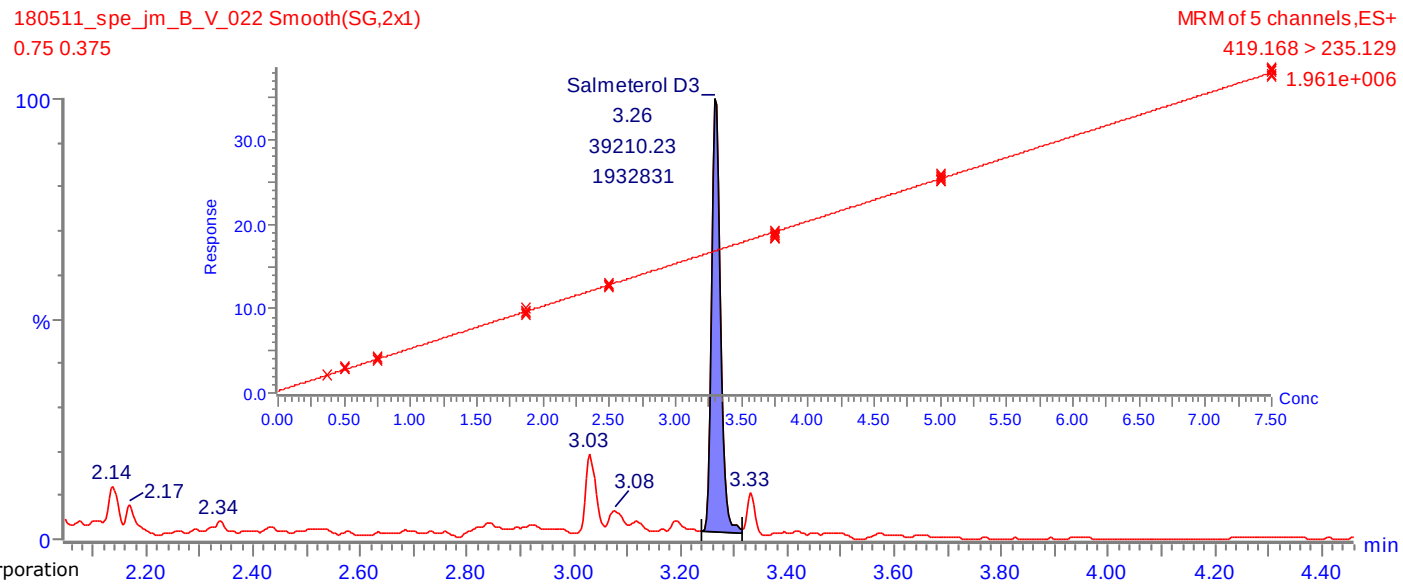


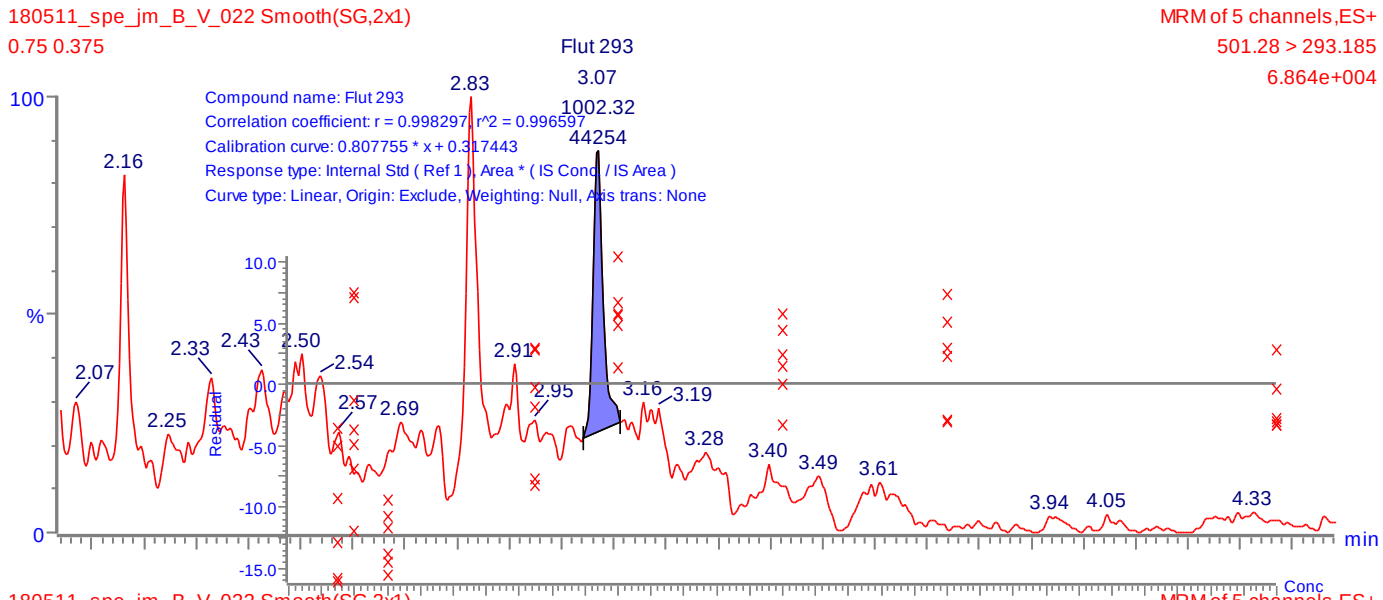
Figure 4. Positive ion LC/MS/MS chromatograms of alprazolam and D5-alprazolam internal standard in protein-precipitated plasma. Top chromatogram: full scan MS chromatogram; middle chromatogram: D5 internal standard MRM analysis (m/z 314 > 286); lower chromatogram: alprazolam MRM analysis (m/z 309 > 281).

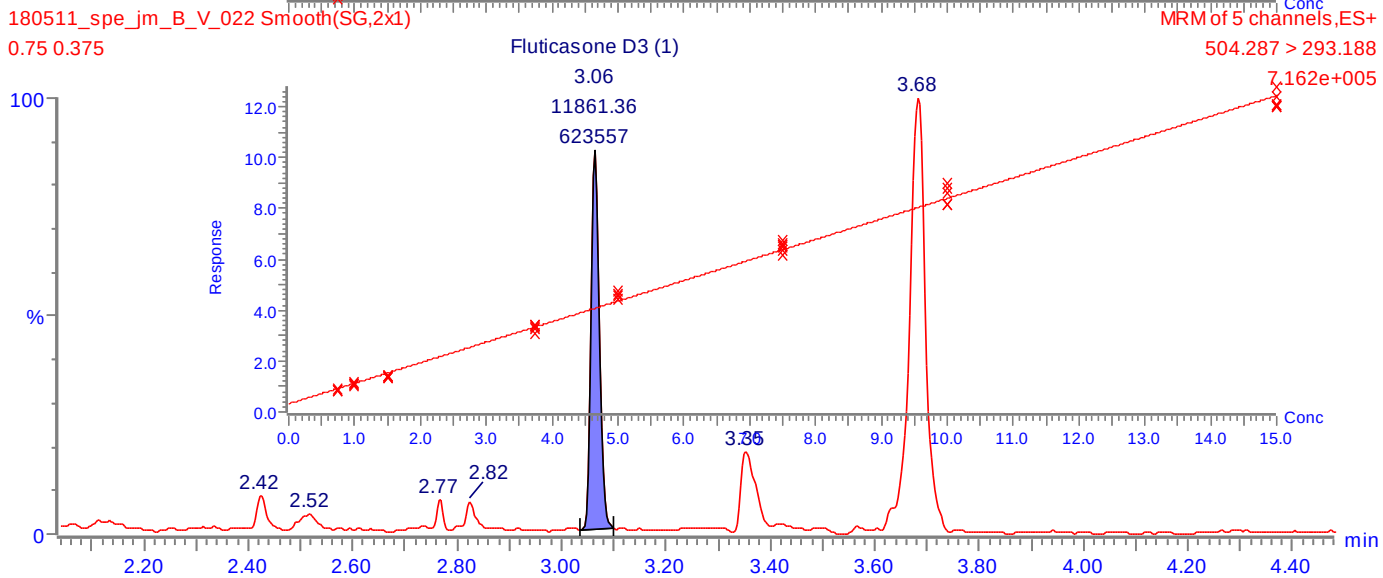
Salmeterol Xinoafate 0.375pg/mL



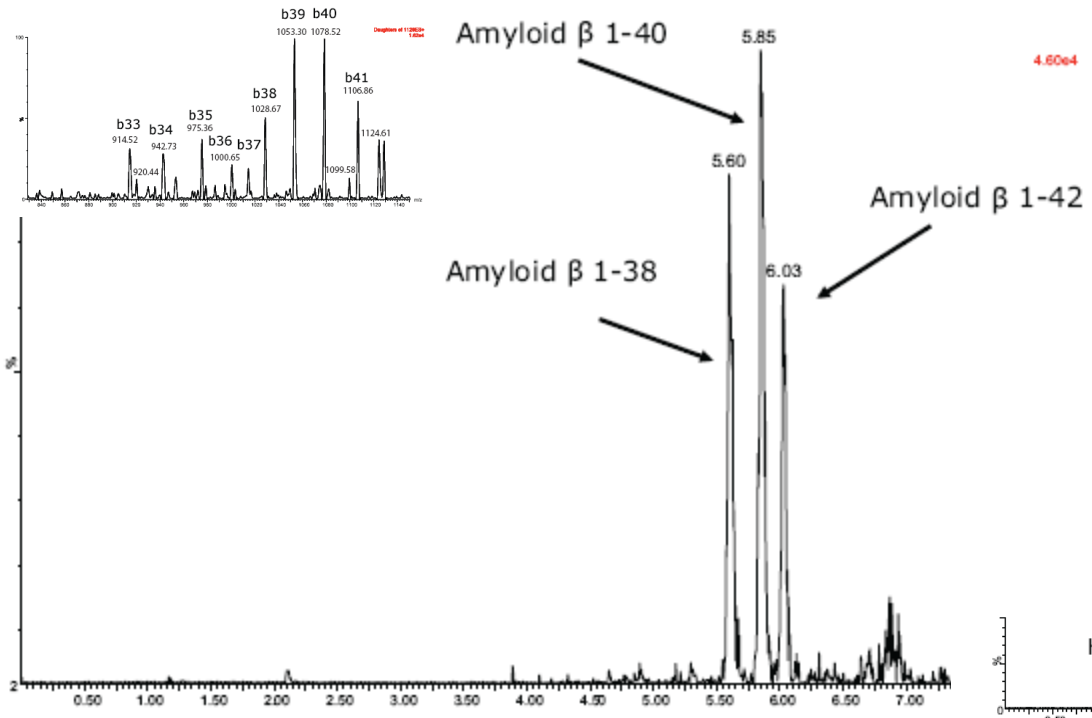


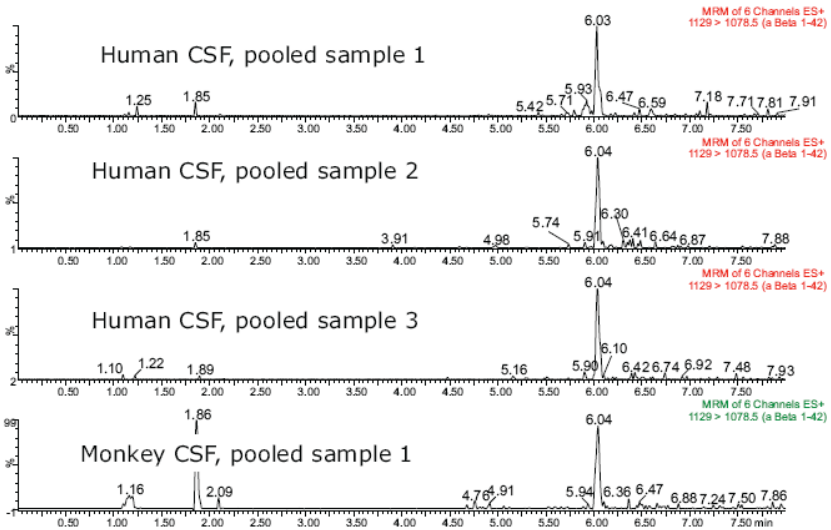
Fluticasone Propionate 0.75pg/mL





Amyloid β Analysis in CSF





Data courtesy of Erin Chambers, Waters

Conclusion

Chromatographic separations and MS detection of complex samples was successfully carried out with pH 10 mobile phase coupled with ESI positive ionization.

Each set of mobile phase conditions lead to the detection of analytes not seen with the opposite pH conditions

The chromatographic performance with pH 10 mobile phases showed increased resolution, sensitivity, and selectivity

Acknowledgements

- Prof. Jeremy Nicholson, Prof. Elaine Holmes, Dr. Derek Crockford, Dr. Robert Plumb, Dr Elizabeth Want (Imperial College London, UK)
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- John Shockcor (Waters Corporation USA)

AstraZeneca

Imperial College London

KING'S College LONDON