# High Throughput - High Temperature LC/ Electrospray-MS

Jennifer A. Townsend and Robert D. Voyksner  LCMS Limited, PO Box 27228, Raleigh, NC, USA 27611  www.lcmslimited.com

## Overview:

**The use of elevated temperatures (75-200C) and zirconia based column packings resulted in LC separations with improved resolution, shorter analysis time and improved electrospray MS sensitivity**

## Introduction:

**The use of zirconia based column packings for LC offers the potential of high throughput and reduced background for LC/MS. These column packings are stable at elevated temperatures, reducing the pressure drop across the column due to the reduction of solvent viscosity thus improving mass transfer. Therefore, at elevated temperatures it should be possible to increase flowrates, reducing analysis time while maintaining the separation efficiency. Secondly, the heated solvent could be beneficial for the nebulization and desolvation steps increasing the efficiency of the formation of electrospray ions via ion evaporation.**

**This presentation will evaluate the use of zirconia based packings, comparing the sample throughput relative to silica based columns of the same dimensions. The influence of column temperature on separation speed, peak shape and electrospray response will be evaluated for a series of compounds of pharmaceutical interest.**

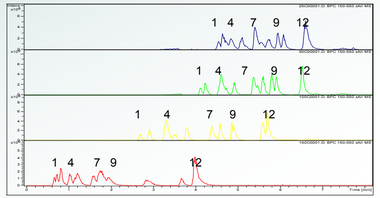
## Methods:

**Zirconia polybutadiene columns and Zorbax C18 columns of 0.5 and 2.1 mm in diameter by 50-100 mm in length with 3 um particles, were evaluated using solvent gradients of water to acetonitrile containing 25 mM ammonium acetate. The zirconia based columns were evaluated over temperatures 20-200C and at flowrates up to 1.5 ml/min. The LC/MS data was generated on the Agilent 1100 LC/MSD Trap. The trap was operated in positive ion electrospray mode. The performance of the High Throughput High Temperature LC/MS [(HT)$^2$ LC/MS] was compared to separations on the silica column at 25C using comparable gradient elution, for a series of compounds of pharmaceutical interest. These compounds included benzodiazepines, sulfa drugs, analgesics and Cytochrome P450 inhibitors.**

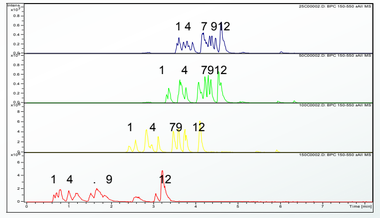
## Results:

**1. Evaluation of temperature and gradient profiles**

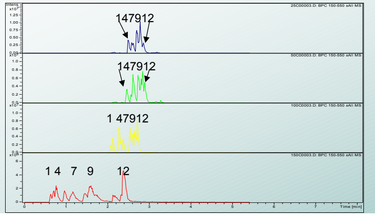
0 -100% B in 12 min




0 -100% B in 6 min



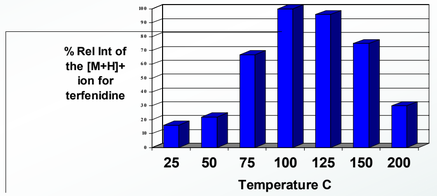

0 -100% B in 2 min.




**Temperatures**
**blue 25 C**
**green 50C**
**yellow 100C**
**red 150C**

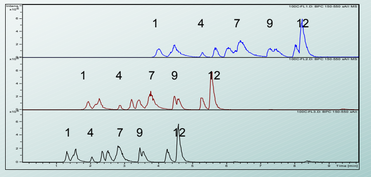
**Elution order**
1. Alprazolam
2. Triazolam
3. Propanolol
4. Diazepam
5. Midazolam
6. Haloperidol
7. Propafenone
8. Desipramine
9. Nortriptyline
10. Chlorpromazine
11. Fluphenazine
12. Terfenidine

**2. Evaluation of ESI signal intensity as a function of solvent temperature.**

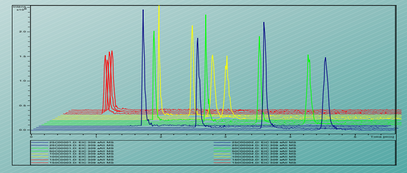



**3. Evaluation of flow rates for the separation of the test mix at 100C for a 0 – 100% B gradient in 12 min.**

250 ul/min
500 ul/min
750 ul/min




**4. Evaluation of temperature (25, 50, 100, and 150 C) and gradient rate ( 0-100% B in 12, 6, 2, and 0.5 minute) for the analysis of alprazolam**



## Conclusions:

1. **Going from 25 C to 100 - 150C in column temperature decreased the analysis time thus increasing the retentive time window (larger peak capacity) for compound elution.**
2. **Electrospray sensitivity increased by a factor of 2-8 at 100C over room temperature operation for the 12 drugs evaluated. It is postulated the superheated solvent aids in nebulization and desolvation steps that must occur in the few millisecond residence time in the atmospheric chamber to form ions by ion evaporation.**
3. **Operation at elevated temperatures permitted separations at 3-4 times higher flowrates than silica columns. The separation efficiency was maintained while analysis time and column re equilibration time was reduced.**
4. **Target compound analysis sensitivity, specificity and speed can be achieved by a combination of elevated temperature operation and solvent composition.**

## Acknowledgements:

• ZirChrom Separations