



## DRUG DEVELOPMENT SOLUTIONS

Part of Alliance Pharma, Inc.

# A step-by-step guide to developing a robust assay in bioanalysis using LC-MS/MS

LC-MS/MS assay development within Bioanalysis is driven by developing accurate, precise and robust methods within the shortest time frame possible. Within our department we have employed a generic protocol to enable a consistent and efficient process to reach these goals.

## LC-MS Conditions

### Initial Screening

Determine: Log D and pKa to assist in initial decision making

### Infusion of analyte into MS

- » Pos/Neg, ESI/APCI
- » M+H or M-H or Adducts
- » Minimise adducts or maximise

### Scouting HPLC Gradients

- » Acidic & Basic & buffered mobile phases for retention and signal:noise (S:N)
- » Gradient for sharper peaks and sensitivity
- » Isocratic for resolution

### Limit of Quantitation

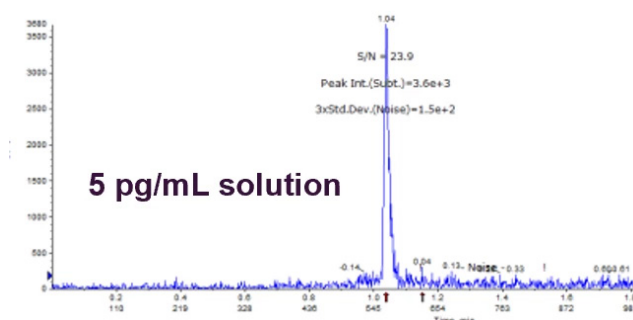
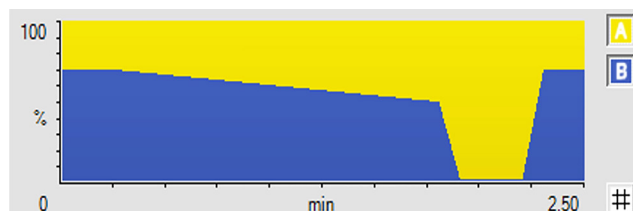
- » Dilution series made in solution
- » Provides an idea of what sort of sample extraction technique is needed
- » S:N of 20:1 to enable an ↑chance it will be suitable in matrix

## GUIDE

CENTRE OF  
EXCELLENCE

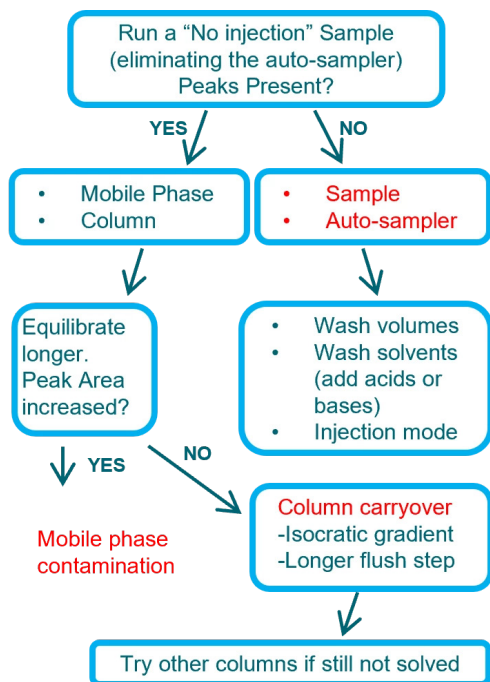
PROTEIN LC-MS

Time	%Organic	%Aqueous
0.00	30	70
0.25	30	70
1.80	50	50
1.90	98	2
2.20	98	2
2.30	30	70
2.50	30	70

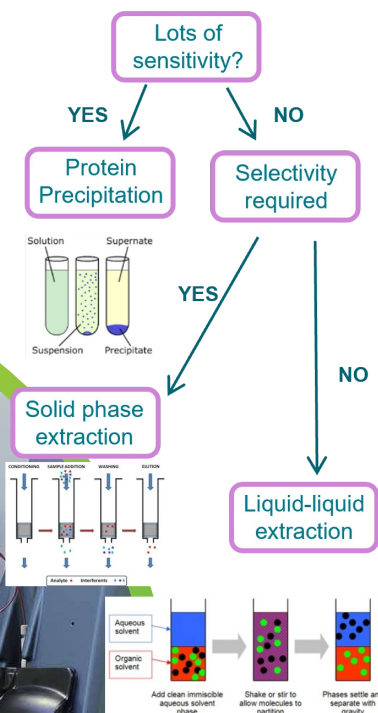


## Carryover

If observed will need to locate the source:



## Sample Extraction

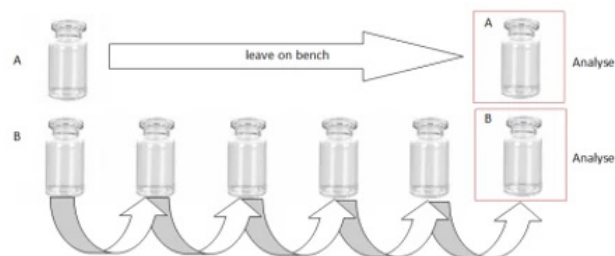


## Binding

### Non-specific Binding

Binding test performed as follows in three types of materials:

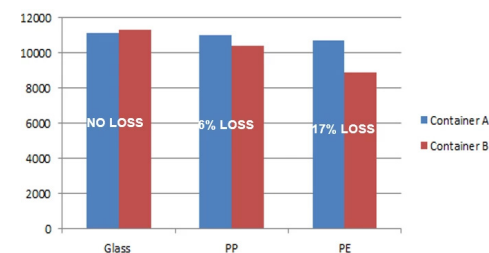
- » Glass
- » Provides an idea of what sort of sample extraction technique is needed
- » S:N of 20:1 to enable an ↑chance it will be suitable in matrix



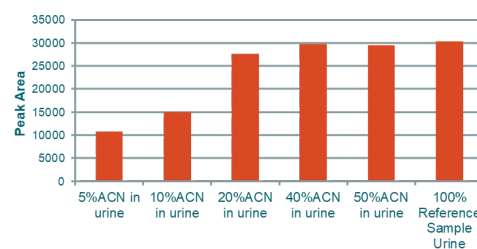
Analyte 1



Analyte 2



### Binding in Urine



## Conclusion

Utilising this generic guide as a starting point for method development of each new compound streamlined our processes and highlights issues at an early stage and creates a solid basis for future troubleshooting.



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