Optimising Sample Throughput in Bioanalytical Workflows

Matt James[1], Tony Edge[1], Geoff Faden[2]

[1] Avantor, Theale, Reading, Berkshire RG7 4PE, UK, [2] MAC-MOD Analytical Inc., 103 Commons Court, PO Box 587, Chadds Ford, PA 19317 USA

1. Background

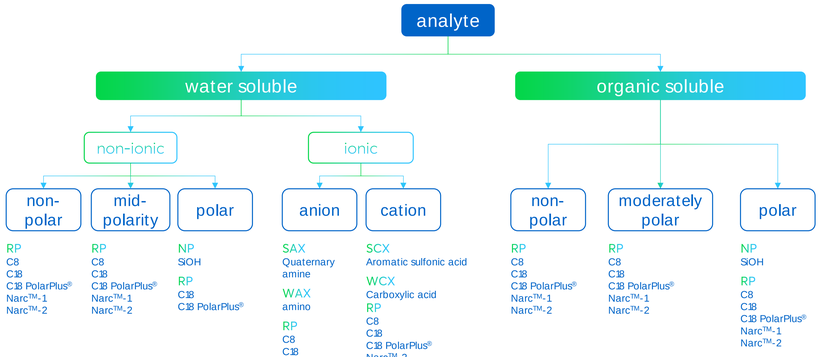
- Sensitive and selective LC-MS analytical protocols benefit from sample preparation.
- For biological samples, different approaches including Protein precipitation (PPP), SLE and SPE can be utilised.
- Differing approaches remove different amounts of matrix components.
- Differing samples will have different matrix components resulting in a variable effect on the detector response.
- Effective removal of matrix components results in robust assays.

2. Which sample prep approach?

- SLE, PPP & SPE all have their advantages:

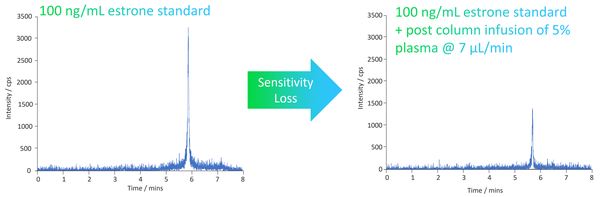
	SLE	PPP	SPE
Selectivity	☺☺☺	☺	☺☺☺☺
Cost	☺☺	☺☺☺☺	☺
Time	☺	☺☺☺	☺
Method development time	☺☺	☺☺☺☺	☺☺
Cleanliness	☺☺☺	☺	☺☺☺☺
Range of applicable analytes	☺☺	☺☺☺☺	☺☺☺☺

- Selection of an appropriate SPE phase, base on analyte physico-chemical properties:



3. Investigating sources of signal suppression

- Matrix components can impact analyte response (matrix effects):
  - Suppression
  - Enhancement
- Demonstration of the influence of matrix effects on the analysis of estrone (MRM 269.1→ 145.0) by LC-MS.
- Not removing the matrix reduces the signal.
- Magnitude of reduction depends on amount of matrix component eluting, which can be highly variable.

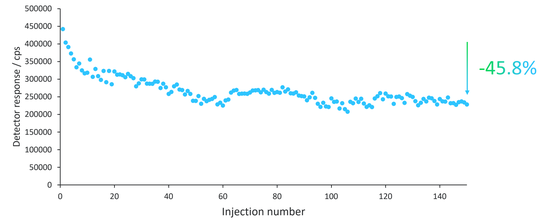


Column: Avantor® ACE® Excel® 2 SuperC18, 100 x 2.1 mm

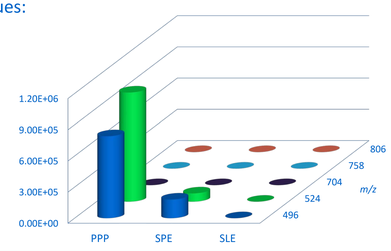
Mobile Phase: A: 20 mM $NH_4CH_3CO_2$ (aq) B: 20 mM $NH_4CH_3CO_2$ in MeOH/H_2O (90:10)

Gradient: 2%B for 1 min, then 2-60%B in 5 mins, Flow Rate: 0.4 mL/min, Injection: 1.0 µL, Temperature: 50 °C

- Impact of 150 repeat injections (1 µL) of a 1:20 dilution of protein precipitated dog plasma on MS response:



- Removal of phospholipid components by different sample preparation techniques:



4. Chromatographic separation & calibration data

- Optimisation of methodology by careful selection of approach, sorbents, load, wash and elution solvents, pH and chromatographic conditions will produce a method that is validatable in accordance with the regulatory guidelines, in this case for Fluticasone.

Sample Preparation

2 µL fluticasone-d5 internal standard was added to 398 µL rat plasma and diluted with 4 volumes of water. The following SPE protocol applied.

SPE Plate:	J.T.Baker®, BAKERBOND® spe C18, 60 mg, 96 well plate
Conditioning:	400 µL of MeCN.
Equilibration:	400 µL of H_2O.
Sample Load:	Load 25 µL of pre-treated sample.
Wash:	Wash with 200 µL water
Elution:	Elute analytes with 200 µL of DCM.
Post Elution:	Evaporate to dryness & reconstitute in start mobile phase.
Analysis:	Eluent analysed by LC-MS/MS

Chromatography

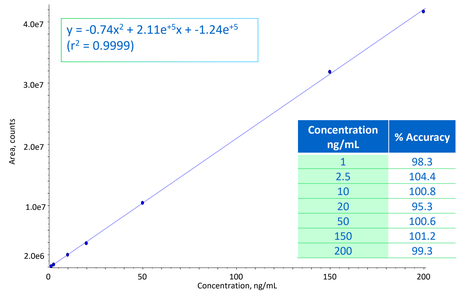
Column:	Avantor® ACE® Excel® 2 SuperC18
Particle Size:	2 µm
Dimensions:	50 x 2.1 mm
Mobile Phase:	A: 0.1% formic acid (aq)
	B: 0.1% formic acid in MeOH

Gradient:

Time (min)	% B
0	45
0.1	45
2.0	95
5.0	95
5.1	45

Flow Rate:	0.4 mL/min
Injection:	5 µL
Column temp.:	45 °C
Detection:	Sciex QTRAP® 6500+ LC-MS/MS system.

Calibration



Concentration ng/mL	% Accuracy
1	98.3
2.5	104.4
10	100.8
20	95.3
50	100.6
150	101.2
200	99.3

QCs

Concentration (n = 5), ng/mL	Mean ng/mL	% Precision	% Accuracy
10	9.9	9.0	99.5
20	19.6	14.1	97.8
80	78.0	2.7	97.5
100	100.5	7.8	100.5

- Data obtained from the SPE-LC-MS/MS assay is excellent.
- Demonstrates importance of optimising the sample preparation and chromatographic conditions to remove adverse matrix effects.

5. Summary and Conclusions

- Different approaches to sample preparation can be employed, with different advantages and disadvantages to each approach.
- Ion suppression can have dramatic impact on assay stability and also the detection limits.
- Matrix components are removed quantitatively different using different approaches.
- Excellent linearity, accuracy and precision demonstrated for an optimised SPE-LC/MS/MS assay.
- Rapid LC-MS/MS separation developed on an Avantor® ACE® Excel® 2 SuperC18.