Chromatography Solutions

Optimising the extraction of oligonucleotides by SPE

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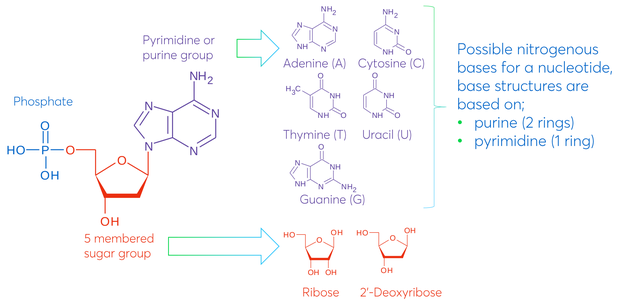
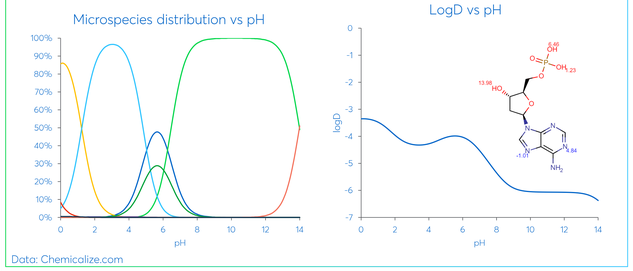
1. Background

- Oligonucleotides are receiving substantial interest from the pharmaceutical market due to enhanced efficacy and lower toxicity in a variety of therapeutic areas.



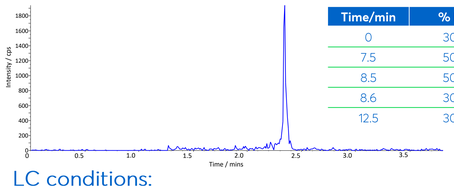
Deoxyadenosine monophosphate:



- For a single nucleotide, the number of possible charge states is very high and the logD plot is very complex, implying that oligos will be incredibly complex. This results in challenges producing a stable assay.
- This poster will look at an approach that will optimise the extraction methodology.

2. LC-MS conditions

- Ion pair chromatography was used for the separation of a series of oligonucleotides, this was based on a previous application note.
- LC analysis of thymidine 20mer:



Time/min	% B
0	30
7.5	50
8.5	50
8.6	30
12.5	30

LC conditions:

Column:	Avantor® ACE® Excel 3 Oligo
Dimensions:	50 x 2.1 mm
Mobile phase A:	15 mM Di-n-butylamine + 25 mM HFIP in H_2O
Mobile phase B:	15 mM Di-n-butylamine + 25 mM HFIP in MeOH
Flow rate:	0.6 mL/min
Temperature:	60 °C
Injection:	10 µL

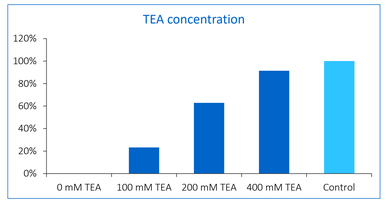
MS conditions:

Source:	ESI
CUR:	20 psi
IS:	-4500 V
TEM:	450 °C
GS1:	50 psi
GS2:	50 psi
DP:	-60 V
EP:	-10 V

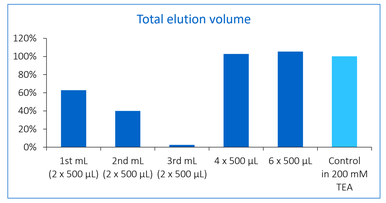
Q1 m/z	Dwell/ms
561.5	150
601	150
627.5	150
646.3	150
704.5	150
671.5	150

3. Optimisation of elution conditions

- Oligonucleotides are negatively charged, and hence a weak anion exchange sorbent (WAX), which has the opposite charge, was chosen as this allowed initial retention and elution by changing the charge state on the sorbent.
- Aqueous samples of thymidine were loaded onto a WAX SPE cartridge and eluted under different conditions. Initial experiments used a loose packed sorbent.



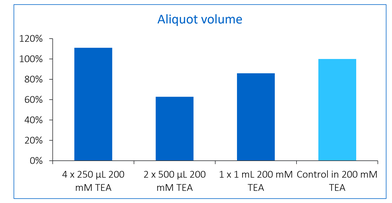
TEA is required to elute thymidine 20mer from the WAX SPE cartridge



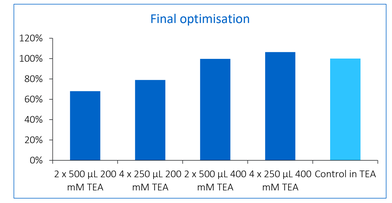
Sequential elution of thymidine 20mer from the WAX SPE cartridge demonstrates that more than one elution step is required

3.(contd.) Optimisation of Elute conditions

- It is evident that optimisation of the elution step is critical to ensure full recovery of the oligonucleotide. Different oligonucleotides may need different elution conditions, but the proposed strategy ensures that this stage can be optimised efficiently.



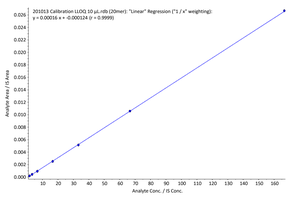
Impact of varying the number of elutions but keeping the same total volume. More elutions gives greater recovery.



Final optimisation of elution step looking at number of elutions and also the concentration of TEA.

4. Aqueous extraction curve

- Optimisation of the elution of an aqueous solution of thymidine 20mer allowed the determination of an extraction curve for aqueous samples. This allows the linearity of the assay performance to be calculated as well as the robustness of the chemistry aspect of the extraction procedure. Salicylic acid was used as an internal standard.



Calibration standard	Peak Area	Concentration (ng/mL)	Calculated Concentration (ng/mL)	Accuracy (%)
L8	9.54E+03	150	159	106.0
L7	2.49E+04	300	299	99.7
L6	5.44E+04	600	577	96.2
L5	1.51E+05	1500	1490	99.0
L4	2.93E+05	3000	2950	98.4
L3	6.14E+05	6000	6010	100.0
L2	1.56E+06	15000	15100	100.0

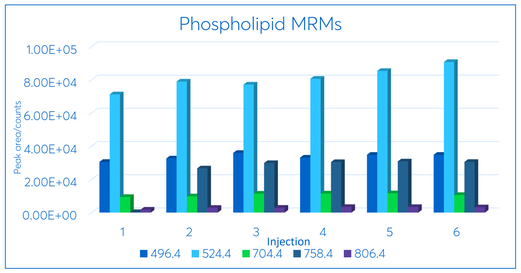
Aqueous extraction curve is linear and the accuracy is very good.

5. Matrix removal

- Initial SPE protocol for plasma analysis - J.T.Baker® BAKERBOND® 30 mg 96 well SPE plates, Diamino (NH/NH2) p/n - 7089-P30

Condition:	1 mL MeOH
Re-equilibration:	1 mL 0.1% FA in Water
Load:	1 mL 10% plasma with 0.1% FA (aq)+ IS
Wash:	Not optimised 1 mL FA in Water 1 mL FA in MeOH
Elute:	4 x 250 µL 400 mM TEA in Water

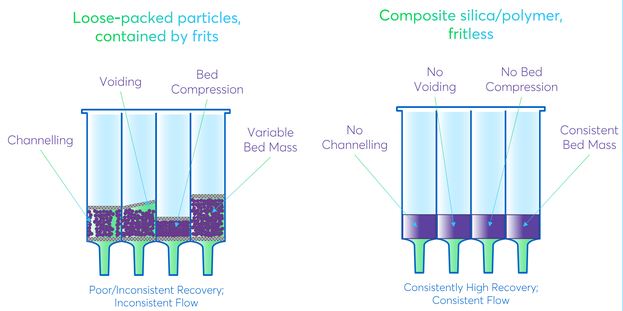
- Method used to process series of plasma samples.
- Residual matrix levels monitored, specifically phospholipid concentration.
- Lack of optimised wash conditions results in increasing build-up of phospholipids on column.



Gradual build-up of phospholipids when plasma extracts are injected

6. Next steps: use of composite SPE materials

- Loose packed SPE has inconsistent flows as shown in the figure. To develop the method further a novel composite material will be employed during method development to reduce the inconsistencies of loose packed SPE formats.

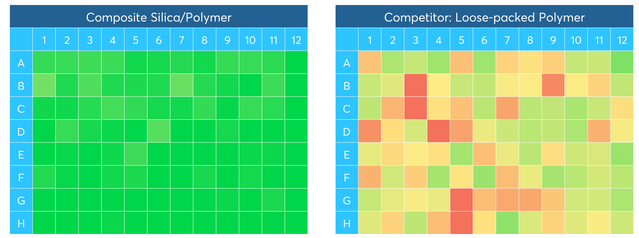


Schematic overview of the challenges associated with loose packed SPE compared to composite SPE

- Recovery from the novel composite material compared to loose packed SPE.
- More uniform flow through the composite material results in much better consistency.

6. (contd.) Use of composite materials

- Comparison of composite and loose packed SPE format, investigating the recovery of a drug from an aqueous solution, highlighting flow inconsistencies between the two formats.



Analyte % Recovery: <85 | 88 | 90 | 93 | 95 | 100

7. Conclusions and Future work

- Optimisation of the extraction process by initially using purely aqueous solutions allows optimisation of the chemistry, without the complexity of a matrix.
- The use of a novel composite form of the SPE media improves the extraction performance reducing the potential for failed sample analysis.
- Subsequent work will look to optimise the digestion and wash steps to ensure that matrix components are effectively removed.