Fortis™ Technologies Ltd.

Application Note

Nucleosides

Introduction

Modified Nucleosides in urine samples can be an indication of carcinogenesis, so analytical methods able to determine these compounds in biological fluids are of high importance. We show an application using UHPLC in order to provide a fast, precise, high resolution separation and quantification of nine modified nucleosides.

UHPLC is particularly suited to this analysis as it is simple, precise, fast and selective, meaning that accurate results can be obtained in minutes rather than hours. High sensitivity is also another improvement that UHPLC tends to offer over traditional HPLC, meaning lower LOD and lower LOQ's can be achieved.

Experimental

Using a standard 12 nucleosides test mixture (Sigma-Aldrich) the performance of two stationary phases was evaluated in order to select the best column for analysis of the modified nucleosides. Next the choice of organic modifier was studied to obtain the best selectivity allowing full resolution of the nucleosides.

Compounds

Sodium formate, 10mg/ml
Pseudouridine, 25ug/ml
Cytidine, 50ug/ml
3-Methylcytidine, 100ug/ml
Uridine, 25ug/ml
1-Methyladenosine, 25ug/ml
2-Thiocytidine, 10ug/ml
5-Methylcytidine, 100ug/ml
7-Methylguanosine, 25ug/ml
2'-O-Methylcytidine, 20ug/ml
Inosine, 25ug/ml
Guanosine, 25ug/ml
Ribothymidine, 50ug/ml

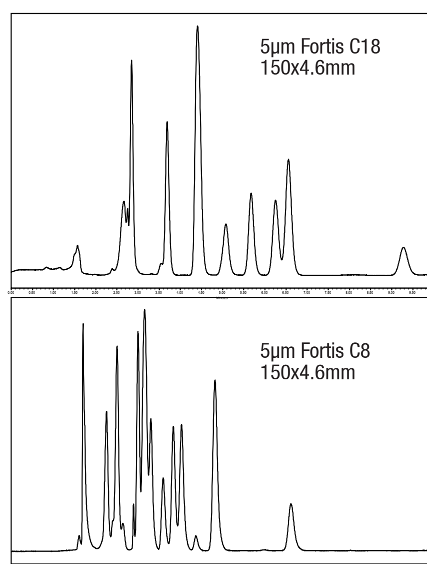

Figure 1. Comparison of C18 vs C8

Figure 1 shows that the Fortis C8 provided resolution of more of the 12 nucleosides when compared to the Fortis C18 phase.

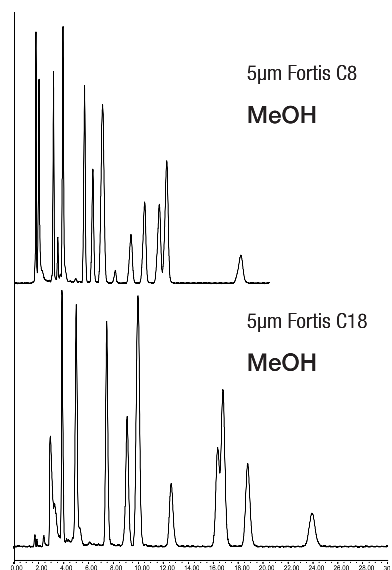

Figure 2. Comparison of organic modifier

From figure 2 we can see that both stationary phases gave improved chromatography with methanol as the organic modifier compared to acetonitrile.

Using Fortis C8 as stationary phase and methanol in the mobile phase we were able to resolve the most nucleosides contained in the mixture in a run time of 20 minutes.

Modified Nucleosides

Using the previous optimum recommended stationary phase and mobile phase conditions the nine modified nucleosides were then analysed:

Cytidine
5-Methylcytidine
7-Methylguanosine
5-Hydroxymethyluridine
Adenosine
Guanosine
5-Methyluridine
Thymidine
N^2-methylguanosine

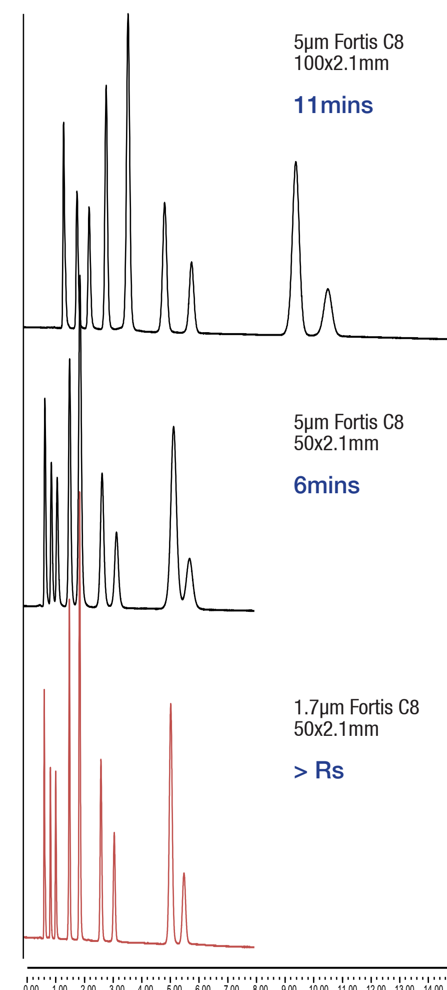

Figure 4. Optimise method

Using a 5µm Fortis C8 100x2.1mm column we first looked at the optimum conditions from the previous analysis, we obtain good resolution of the 9 nucleosides. Optimisation by shortening the column and increasing the flow rate removed 5minutes from the run time, however we start to compromise the resolution.

Results

Moving to a 1.7µ column returns the resolution and shows that we can decrease the run time further due to the increased resolution.

The final fully optimised method is:

Column: 1.7µm Fortis™ C8 30 x 2.1 mm
p/n F08-020201
Mobile phase
95:5 Water : MeOH
Flow Rate: 0.3ml/min
Temp: 25°C
Detection: 254nm

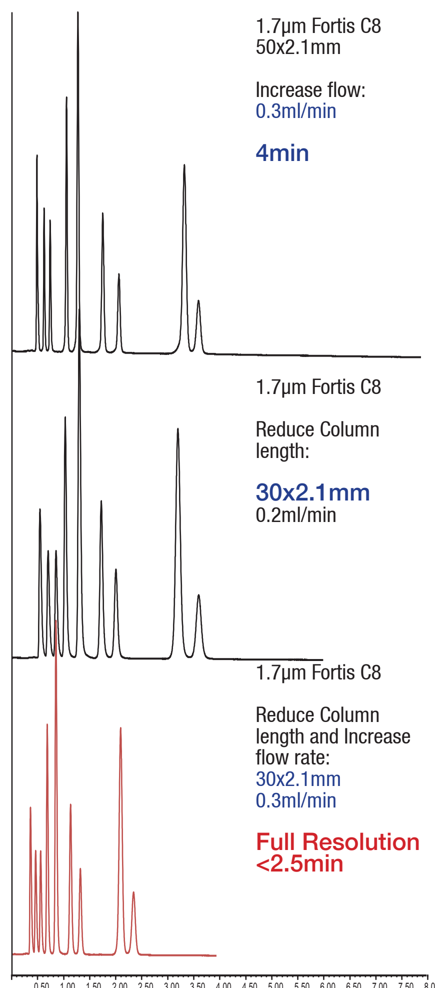

Figure 5. Optimise in UHPLC

Conclusion

We have shown a logical method development of 9 modified nucleosides which are crucial in the detection of carcinogenesis.
We look for the choice in stationary phase and organic modifier in terms of their ability to distinguish between closely related nucleosides. Then we try to increase the speed of analysis whilst still retaining a high level of reoslution between the analytes.

Fortis™ C8 showed the most resolution of the nucleosides with a Water : MeOH mobile phase.
Using the 1.7µm particle UHPLC column in a short format allowed us to get the whole analysis cycle down to less than 2.5minutes. The fast analysis allows us to keep up with the need for high efficiency separations in a short period of time. More samples through-put will be achieved allowing more screening for clinical laboratories.

1.7um Fortis™ is a trademark of Fortis Technologies. All columns are original manufacturers own.

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