An Evaluation of a Multi-Mode Stationary Phase for HPLC Separations Using a Multi-Component Test Mix

Denise Logue[1], Dave Lugo[1], Cesar Ramirez-Molina[1] and Dave Mallett[2]
[1]Translational Discovery, EpiNova DPU, II CEDD, GSK & [2]R CEDD DMPK, GSK

Introduction

Within Translational Discovery, rapid sample analysis is paramount, with minimal time available for method development. To aid the turnaround, the use of a generic HPLC column applied to multiple applications is advantageous. The suitability of different HPLC columns for generic applications can be compared using a standard test mix.

C18 columns used in reverse phase chromatography are largely limited to non-polar compounds; this can be problematic for drug metabolites as these are often ionic. A mixture containing both acidic and basic analytes will often require different columns and/or gradients for separation.[1]

A novel stationary phase has been proposed for this type of separation. The Scherzo SM-C18™ column[1] is claimed to be the "World's first multi-mode octadecyl silane (ODS) column." Containing ionic, cationic and ODS ligands, the column is designed to perform in normal and reverse phase modes in addition to the separation of ionic analytes.[1]

Objective

- To develop a chemically diverse test mix; and utilise this tool in the evaluation of the applicability of the Scherzo SM-C18™ for generic HPLC separations in a DMPK laboratory.

Methods

A pool of 30 common test mix compounds were preselected from literature data. From this pool, 10 representative compounds were selected to form the final test mix (**Table 1**), based on their chemical properties.

Table 1: Chemical Properties of the test mix

Compound	MW (g)	Classification	cLogP
Nicotinic Acid	123.1	Acid	0.4
Tryptophan	203.2	Zwitterion	-1.3
Bromoguanosine	362.14	Base	-2.27
Labetalol	328.4	Zwitterion	2.7
Aspirin	180.2	Acid	1.4
Fexofenadine	501.66	Zwitterion	5.6
Reserpine	608.7	Base	3.2
SB243213A	428.42	Base	5.3
Ibuprofen	204.1	Weak Acid	3.6
Penicillin V	350.39	Zwitterion	1.4

Separation of the test mix was optimised on a Waters Acquity UPLC™ system coupled to a photodiode array UV Detector. Data was captured in the 210 – 350nm range. A generic 1 minute separation method was initially used, with the 10 test mix compounds being added sequentially to the analyte mixture. The gradient was modified as necessary to achieve the optimum resolution for all 10 compounds.

(Mobile phase A was HPLC water containing 0.1% (v/v) formic acid; Mobile phase B was acetonitrile containing 0.1% (v/v) formic acid).

Results & Discussion

A 5 minute chromatographic method was selected for the separation of the test mix on the 5cm Scherzo SM-C18™ column (**Figure 1**). The test mix was then analysed on an Ascentis Express C18 column (**Figure 2**), and an Agilent Zorbax SB-CN column (**Figure 3**). In each figure, 10 chromatograms have been overlayed to highlight the reproducibility of the separation method. It was observed that separation of the 10 compounds was possible using the Scherzo SM-C18. However, when using a standard C18 column the 10 compounds were not fully resolved.

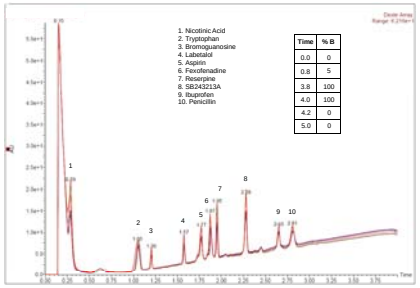

Figure 1: 10 overlayed chromatograms of a typical test mix separation on the 5cm Scherzo SM-C18 column

Separation of all 10 compounds using the Scherzo SM-C18™ column. Note: nicotinic acid is not well resolved from the solvent front.

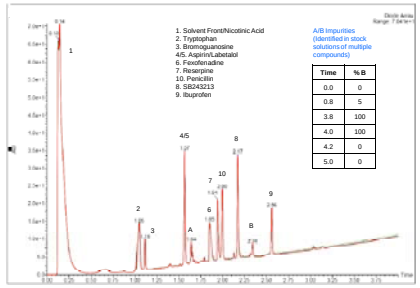

Figure 2: 10 overlayed chromatograms of a typical test mix separation on the 5cm Ascentis Express C18 column

Separation of the 10 compounds on the standard C18 column used in the DMPK laboratory. Note: All 10 compounds are not fully resolved.

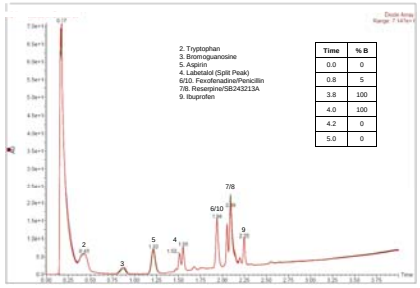

Figure 3: 10 overlayed chromatograms of a typical test mix separation on the 5cm Agilent Zorbax SB-CN column

A typical cyano column is not capable of separating all 10 compounds under these generic separation conditions.

Mobile phase composition was investigated by using different combinations of ammonium formate and formic acid additives. The results of these experiments are summarised in **Table 2**. No significant improvements were made to the original separation, therefore, all further experiments were performed under generic mobile phase conditions.

Table 2: Summary of Experiments Conducted Under Different Mobile Phase Conditions

Conditions	Mobile Phase A	Mobile Phase B	Results Observed
Generic (Acid)	H_2O + 0.1% (v/v) formic acid (pH 2)	MeCN + 0.1% (v/v) formic acid (pH 5)	Best overall separation – one peak present for each compound.
Buffer Only	H_2O + 10mM NH_4COOH (pH 6)	90% MeCN (aq) + 10mM NH_4COOH (pH 6)	Poor resolution of nicotinic acid from solvent front. Co-elution of SB243213A, penicillin & ibuprofen. Peak broadening observed.
Buffer + Acid	H_2O + 10mM NH_4COOH + 0.1% (v/v) formic acid (pH 3)	90% MeCN (aq) + 10mM NH_4COOH + 0.1% (v/v) formic acid (pH 4)	Poor resolution of nicotinic acid from solvent front. Co-elution of SB243213A, penicillin & ibuprofen.
No Additives	H_2O Only (pH 6)	MeCN Only (pH 5)	Compounds elute in same order as observed for generic mobile phase conditions. Poor resolution of nicotinic acid from solvent front.

Additional work on a 10cm Scherzo SM-C18™ column was carried out. As initial data looked promising, the HPLC method was optimised and extended to 9 minutes and separation of the 10 compounds was improved (**Figure 4**). Nicotinic acid had been separated from the solvent front, compared to the 5cm Scherzo SM-C18™ column (**Figure 1 vs. Figure 4**).

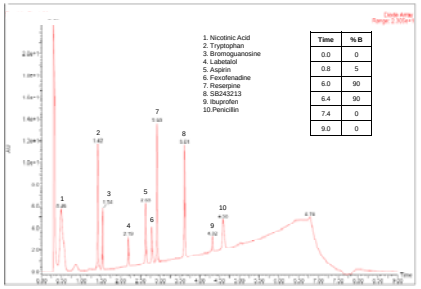

Figure 4: A typical test mix separation on the 10cm Scherzo SM-C18 column using a 9 minute gradient

Conclusions & Further Work

Separation of the test mix was optimised on the 5cm and 10cm Scherzo SM-C18™ columns, using a generic gradient and typical DMPK mobile phases. The SM-C18™ is more effective in separating a mixture of analyte chemistries, than a standard C18 column and a cyano column. This has the potential for rapid analysis of samples where compounds with different physicochemical properties are quantified in addition to the analyte of interest. Work is currently ongoing to test the column robustness by LC-MS/MS analysis of the test mix in blood, and to identify further possible drug metabolism applications for the Scherzo SM-C18™ column.

References:

1. Imtakt Technical Report TR03A, '*The world's first multi-mode ODS column*', Imtakt Corp, Japan, 2009