



Evaluation of C18 Sub 2 Micron Particle Columns for UPLC Analysis of Drug-like Molecules

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Overview

- An evaluation of UPLC sub 2 micron C18 columns was done to determine if a superior product exists for open-access LCMS on drug-like molecules
- A range of columns were analyzed using a 1.5 minute linear gradient with water and acetonitrile plus 0.1% formic acid
- Columns were evaluated based on elution, retention, resolution, peak shapes and pressure
- The system suitability test mix used for the evaluation is a combination of neutral, acidic, and basic compounds
- Of the eight columns evaluated only three were comparable to the Waters Acquity BEH C18 column, our current preferred UPLC column
- Further investigation will be done to determine robustness of the three comparable columns

Introduction

Over the last few years we have converted our high throughput open-access (OA) LC-MS systems to the Waters Acquity UPLC® using the 1.7 micron C18 BEH column. Initially, comparable columns for the UPLC were limited. Recently several competitors have released sub 2 micron columns to be used with the ultra high pressure LC systems. An evaluation was required to determine if a product superior to the Waters 1.7 micron C18 BEH column exists. This experiment included the following sub 2 micron C18 columns.

Columns:

- Waters Acquity 1.7µm BEH C18
- Restek 1.9µm Pinnacle DB C18
- Sepax GP-C18 1.8µm
- Agilent Zorbax 1.8µm RRHT SB-C18
- Grace-Davidson 1.5µm Vision HT C18
- Grace-Davidson 1.5µm Vision HT C18 High Load
- Thermo 1.9µm Hypersil Gold C18
- Macherey-Nagel 1.8µm Nucleodur C18 Gravity
- ES Industries 1.8µm Epic C18

Experimental

For the evaluation of the sub 2 micron columns our current open access standard method and equipment were used. The experimental conditions were as follows:

Waters Acquity –SQD

PDA scanning 210nm to 350nm, sampling rate of 40 points/sec

Solvent A: Water + 0.1% v/v formic acid

Solvent B: Acetonitrile + 0.1% v/v formic acid

Gradient:

Time (min)	Flow (mL/min)	%A	%B	Curve
initial	1.0	95.0	5.0	initial
1.1	1.0	0.0	100.0	6.0
1.5	1.0	0.0	100.0	6.0

Column Temperature: 40°C

Injection volume: 0.5µL

Injection mode: Partial loop with needle overfill

Each column was equilibrated for 15 minutes at 100% Solvent B. After the column had equilibrated, three injections of a seven component system suitability test mix (SSTM) were run. The third injection for each column was compared.

The seven component SSTM is a combination of neutral, acidic and basic compounds. The SSTM was designed to test the retention of early eluting compounds as well as ensure elution of a rather lipophilic compound.

Each column was evaluated for:

- Retention of bromoguanosine
- Retention and peak width of amitriptyline
- Ability to Resolve amitriptyline and 4-chlorocinnamic acid
- Elution of di-octyl phthalate
- Peak shapes and resolutions
- Maximum backpressure

Results

Figure 1: UV chromatograms of columns that pass minimum criteria

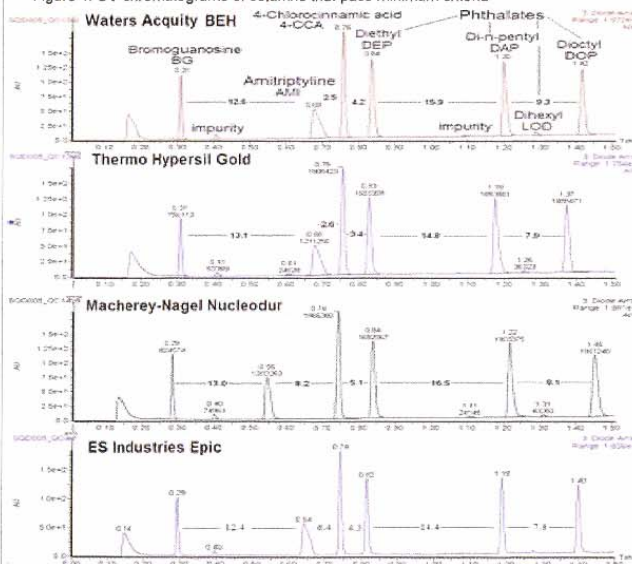
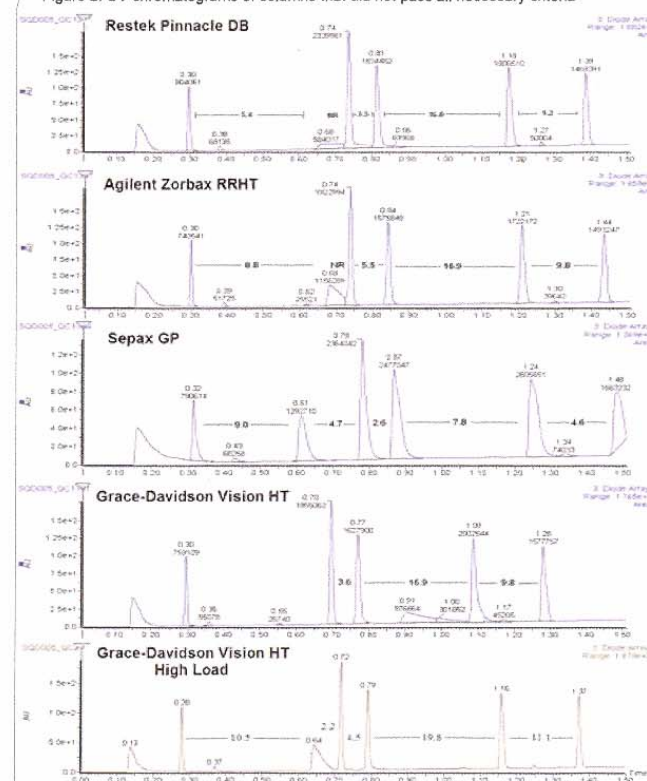


Table 1: Summary of Tailing, Resolution between amitriptyline and 4-chlorocinnamic acid, and observed max pressures

Supplier	Tailing (AMI)	Resolution Ami to 4-CCA	Max Pressure (psi)
Water's 1.8µm	1.9	2.5	10194
Machery 1.8µm	1.4	8.2	9458
Thermo 1.9µm	2.1	2.6	7250
ES Ind. 1.8µm	2.1	6.4	11828
Agilent 1.8µm	3.9	Not resolved	8400
Grace HT 1.5µm	4.4	N/A	11650
Grace HL 1.5µm	3.1	2.2	11447
Restek 1.9µm	4.6	Not resolved	10215
Sepex 1.8µm	1.6	4.7	10206

Figure 2: UV chromatograms of columns that did not pass all necessary criteria



Conclusion

The evaluation of sub 2 micron columns for use with UPLC had mixed results. Four columns did not pass the minimum criteria for this initial evaluation. The Sepax GP column had issues with peak shape and tailing that prevented complete elution within the 1.5 minute method. The Restek Pinnacle DB, Agilent Zorbax RRHT, and Grace Vision HT retained the basic component for too long affecting resolution and elution order. While the Grace Vision HT High Load column did exhibit the best resolution of the phthalates in the test mix a greater resolution between the amitriptyline and the 4-chlorocinnamic acid is desired for our purpose. Highlighted in Table 1, the columns with decreased tailing of the amitriptyline prove to be most desirable for an open-access environment that runs a large number of basic compounds. Of the eight columns evaluated only three columns merit further analysis. It is my intention to test out the ES Industries Epic, Macherey-Nagel Nucleodur, and Thermo Hypersil Gold columns on an open-access Acquity in order to determine the robustness and lifetime of each column. Once the durability of each column is determined I will be able to draw a clear conclusion of the value of each column based on cost and lifetime.