Agela Technologies

Systematic Method Developments for Analysis of Highly Water-Soluble Compounds by HPLC

It is known that highly water-soluble compounds have often found difficulties in HPLC analysis, such as lack of retention, bad peak shape or inconsistency. Many recent HPLC stationary phase developments have aimed at providing the solutions. However, it has been proven that no single stationary phase may resolve all. Agela Technologies has developed a series of stationary phases and provides a development scheme to help chemists get a solution for the analysis of highly water-soluble compounds. This application will demonstrate such a method development scheme.

Instrumentation and Methods

Instrument: Shimazu 10A,
Reagents: All solvents and reagents were purchased from either Honeywell (B&J) or Sigma-Aldrich.
HPLC Conditions: Conditions for each method are listed under each chromatogram.

Discussion

Venusil AQ C18 is a slightly-polar C18, a versatile high aqueous reverse phase with broad pH suitability £®1.5-9.0£© and can be the first option for most of HPLC applications. Venusil ASB C18 (0.8–7.0) and Venusil HILIC (2–8) are good supplementary options with higher retention for polar compounds and unique selectivity. Venusil ASB C18 are non-end capped polar C18 columns and good for low pH conditions. Venusil HILIC are unique hydrophilic interaction columns with strong hydrophilic and neutral functionality and are the most retentive columns among the three types.

In general, the method development starts with a Venusil AQ C18 column and a mobile phase that contains a mixture of MeOH or acetonitrile with an aqueous acidic buffer solution (pH=2–5). The retention is usually increased by reducing the organic solvents. In the cases that enough retention but not enough resolution is obtained, one may chose to adjust mobile phase ratio or pH, or chose to replace the column with a Venusil ASB C18 or Venusil HILIC for an alternative selectivity. Figure 2 shows a HPLC separation of 4 organic acids on a Venusil AQ C18 column in a 100% aqueous mobile phase at pH=2.

In the cases that sufficient retention cannot be obtained even with 100% aqueous mobile phase, the following options can be considered.

For acidic compounds, one may replace AQ C18 with a ASB C18 column and adjust pH to 1–2 to reduce the compounds' solubility. One may also use Venusil HILIC to get even higher retention and different selectivity. When a Venusil HILIC is used, more polar compounds will elute later than less polar, in contrast to ASB C18 or AQ C18 columns.

For basic compounds, one may stay with the AQ C18 columns while adjusting the pH to 9, or use Venusil HILIC to replace AQ C18 at lower pH (2–7). In one experiment, sufficient retention could not be obtained for validamycin on a AQ C18 column even with a 100% aqueous mobile phase. Instead Venusil HILIC column gave the validamycin raw material a very good separation at pH=2 in a HILIC mode. See Figure 3 for the chromatogram and conditions.

For neutral compounds, the change of pH will have limited effects on retention. Replacement of the AQ 18 with either ASB C18 or Venusil HILIC will be needed.

Venusil HILIC will be a good choice if a desired retention or resolution cannot be obtained on AQ C18 or ASB C18 because of its unique selectivity and the highest retentivity.

The series of new high aqueous reverse phase and HILIC columns from Agela Technologies along with this method development scheme will make the separation of highly water soluble compounds much easier.

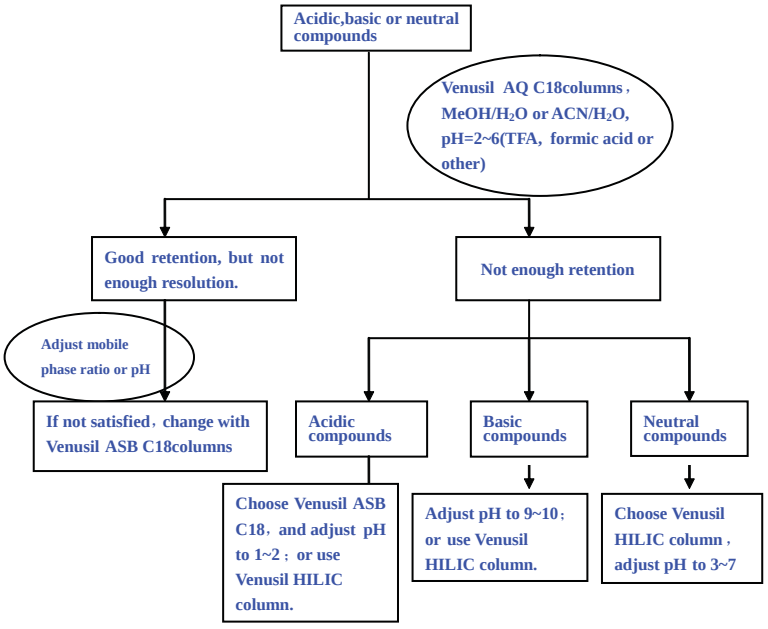

Figure 1: Method development scheme.

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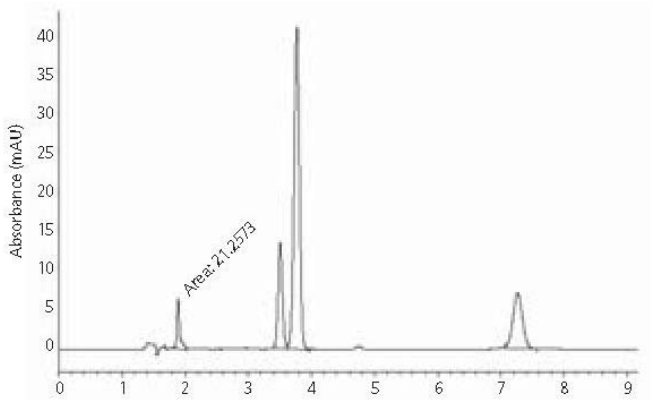

Figure 2: HPLC Separation of Organic Acids
Sample: Vitamin C, Malonic acid, Lactic acid, Citric acid
Column: Venusil AQ C18, 4.6 ×150 mm, 5μm
Mobile phase: 20 mmol phosphate buffer, pH 2.0
Flow rate: 1.0mL/min
Detection: UV 210 nm
Temperature: ambient

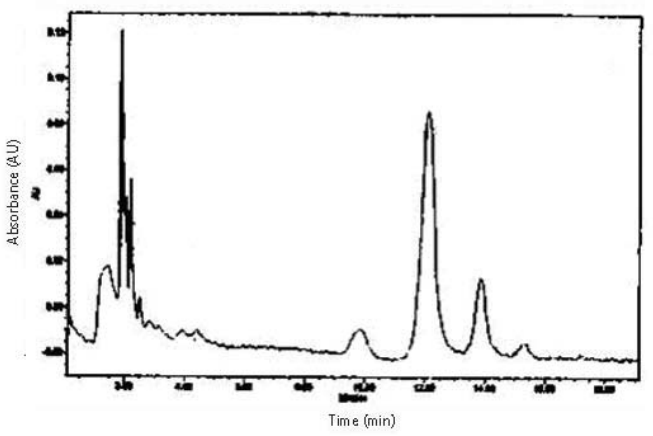

Figure 3: HPLC Analysis of Validamycin Raw Products
Column: Venusil HILIC ,4.6 x 250 mm, 5μm
Mobile phase: ACN/0.1%TFA 85–40% in 30 min
Flow rate: 1mL/min
Detection: UV 210 nm
Temperature: ambient

Ordering Information

Specific Surface 380m²/g, Pore Size100Å

Dimension (mm)	Particle Size (μm)	Venusil AQ-C18	Venusil HILIC
2.1×30	5	VA950302-0	VH950302-0
2.1×50	5	VA950502-0	VH950502-0
2.1×100	5	VA951002-0	VH951002-0
2.1×150	5	VA951502-0	VH951502-0
4.6×50	5	VA950505-0	VH950505-0
4.6×100	5	VA951005-0	VH951005-0
4.6×150	5	VA951505-0	VH951505-0
4.6×250	5	VA952505-0	VH952505-0
4.6×10 4/pk	5	VA950105-0	VH950105-0
2.1×30	3	VA930302-3	
2.1×50	3	VA930502-0	
2.1×100	3	VA931002-0	
2.1×150	3	VA931502-0	
4.6×50	3	VA930505-0	
4.6×100	3	VA931005-0	
4.6×150	3	VA931505-0	
4.6×10 4/pk	3	VA930105-0	

Specific Surface 200m²/g, Pore Size150Å, Particle Size 5μm

Dimension (mm)	Venusil ASB-C18	Venusil ASB-C8	Venusil ASB-Phenyl
2.1×30	VS950302-0	VS850302-0	VS650302-0
2.1×50	VS950502-0	VS850502-0	VS650502-0
2.1×100	VS951002-0	VS851002-0	VS651002-0
2.1×150	VS951502-0	VS851502-0	VS651502-0
4.6×50	VS950505-0	VS950505-0	VS650505-0
4.6×100	VS951005-0	VS851005-0	VS651005-0
4.6×150	VS951505-0	VS851505-0	VS651505-0
4.6×250	VS952505-0	VS852505-0	VS652505-0
4.6×10 4/pk	VS950105-0	VS850105-0	VS650105-0

Specific Surface 45m²/g, Pore Size300Å, particle Size 5μm

Dimension (mm)	Venusil ASB-C18	Venusil ASB-C8
2.1×30	VS950302-T	VS850302-T
2.1×50	VS950502-T	VS850502-T
2.1×100	VS951002-T	VS851002-T
2.1×150	VS951502-T	VS851502-T
4.6×100	VS951005-T	VS851005-T
4.6×150	VS951505-T	VS851505-T
4.6×250	VS952505-T	VS852505-T
4.6×10 4/pk	VS950105-T	VS850105-T

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