Organic Acid Analysis HPLC Columns

Chromatography Application Notes

For over 30 years, Concise Separations has supplied HPLC products to provide customers with solutions for their separation needs. We specialize in polymeric technologies used in a wide variety of HPLC columns, solid phase extraction products, analytical guard columns and cartridges, guard discs, and bulk polymers for purification and sample preparation applications. By providing consistent reliability and timely delivery of high quality, long-lasting products, we have established the Concise Separations Chromatography product line as a mainstay in quality control methods worldwide. We pride ourselves in working closely with our customers to maintain the type of quality and service they need to meet their critical analytical requirements. Through customer collaborations we have developed new methods and applications. In this issue, we highlight some new methods developed for customers using our Organic Acids Analysis HPLC columns.

Introduction

Coregel Columns for Organic Acid Analysis

Ion exclusion is the preferred method for the separation of weakly ionizable species such as organic acids and alcohols. Concise Separations supplies a broad range of columns that provide varying efficiencies and selectivities for the separation of weak acids by ion exclusion.

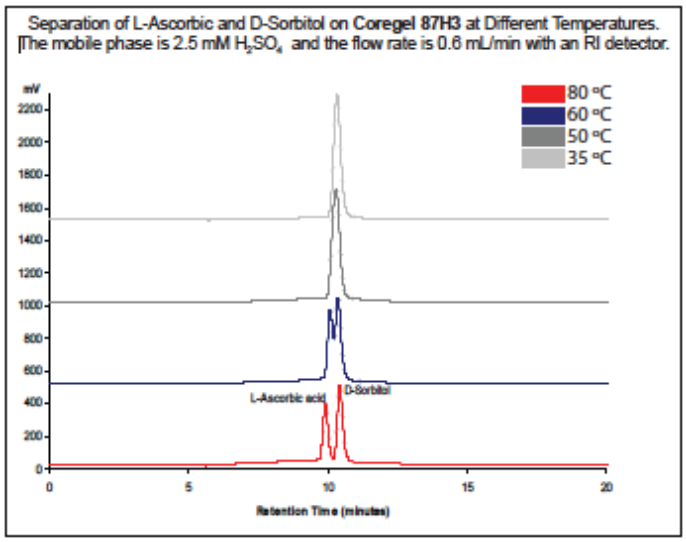
The polymeric packings employed with ion exclusion are totally sulfonated polystyrene divinylbenzene (PS/DVB) copolymers. By totally sulfonating the polymer, the bead behaves as though it is a negatively charged sphere. This charged sphere is referred to as a Donnan membrane. Species that have a negative charge are repelled from the negatively charged membrane, while uncharged species are allowed to enter the sphere and adsorb onto the beads. The mobile phases employed with ion exclusion are low concentration acids, such as 5 mM sulfuric acid.

R-COO- + H+ <——> R-COOH
(repelled) (retained)

This equilibrium is regulated by the acidic dissociation constant (pKa) of the organic acid or alcohol. Therefore, species are analyzed by ion exclusion and generally elute according to their pKa.

Temperature Effect

By far the most powerful tool used to influence relative retention of compounds on Concise Separations ion-exclusion columns is temperature. For polymeric columns, a column oven is usually required for optimum results, and also to minimize the pressure limitations commonly found with other polymeric packings. By manipulating temperature, in combination with eluent strength, and column types (polymer cross-linkage), an analyst can greatly enhance species separation:



Features

Coregel columns are packed with chemically resistant polymeric polystyrene divinylbenzene copolymers varying in percent cross-linkage and particle sizes. Coregel columns provide the following benefits compared to silica based columns:

- Stable in the pH range of 0 to 14
- Stable at high temperatures up to 90 ºC
- Consistent performance through numerous sample injections (depending on sample preparation, instrument maintenance, and the use of guard systems)
- No need for gradients for sample analysis due to the use of simple dilute acid — allowing the use of universal detectors such as refractive index (RI) detectors
- Eliminates the need for high cost solvents (including waste disposal)
- Eluent serves as a self-regenerating cleaning solution and does not degrade the column

How to Choose a Column for Your Sample

In choosing the best column for your application, there are several factors to consider:

- Resolution of peaks of interest
- Analysis time
- Selectivity (elution order of peaks)
- Durability

In general, the **Coregel 87H3** is a good starting point when considering a Concise Separations organic acids analysis column since it combines good resolution of many common organic acids, with high durability. However, the many combinations of polymer cross-linkage, particle size and column sizes offered by Concise Separations allow customers to choose a column with features most important to maximize their separation needs.

Another valuable tool for selecting a column is to use retention charts of many common organic acids. Due to the abundance of organic acids, sugars, and alcohols, and the innumerable possible combinations of the compounds in mixtures, it is impossible to have a totally comprehensive retention chart to conclusively guide a chemist through all applications. Since we stock most of the common organic acids, sugars and alcohols in our laboratory, we frequently will generate a sample chromatogram of your particular sample mixture, or will actually run your sample to ensure that our columns will separate your sample. Please contact Concise Separations for any questions regarding column selection.

Concise Separations Offers More Column Options

Concise Separations has a wider variety of polymeric HPLC columns than our competitors. The vast majority of columns on the market for ion-exclusion analysis of organic acids utilize an 8% cross-linked polystyrene divinylbenzene copolymer.

The chart below is a general guideline for choosing your Concise Separations column:

RESOLUTION (HIGHEST TO LOWEST)	ANALYSIS TIME (FASTER TO SLOWER)	SELECTIVITY (HIGHER TO LOWER)	DURABILITY (MORE TO LESS)
Coregel ION 300	Coregel USP L17	Coregel ION 300	Coregel 107H
Coregel 64H	Coregel 87H1	Coregel 64H	Coregel 87H3
Coregel WA1	Coregel ION 310	Coregel ORH 801	Coregel 87H1
Coregel 87H3	Coregel ARH 601	Coregel WA1	Coregel WA1
Coregel ORH 801	Coregel ORH 801	Coregel 87H3	Coregel ORH 801
Coregel 107H	Coregel 107H	Coregel 107H	Coregel ION 310
Coregel ARH 601	Coregel 87H3	Coregel ARH 601	Coregel USP L17
Coregel ION 310	Coregel WA1	Coregel ION 310	Coregel ARH 601
Coregel 87H1	Coregel 64H	Coregel 87H1	Coregel 64H
Coregel USP L17	Coregel ION 300	Coregel USP L17	Coregel ION 300

Although the 8% cross-linked polymeric columns provide an excellent separation of many typical samples containing organic acids, sugars and alcohols, Concise Separations has determined that by varying the cross-linkage and particle sizes of the polymers, certain separations are enhanced tremendously. Although ion-exclusion of organic acids is the main separation mechanism for samples, the size exclusion limit of the polymers is controlled by the percent cross-linking. The additional size exclusion mechanism for Concise Separations columns provide unique selectivity for some components. For maximum efficiency, Concise Separations also provides columns with smaller particle sizes and tighter size distributions than the typical industry standard of 9 micron particles. By providing additional choices, Concise Separations allows you to maximize separations in terms of speed, selectivity and resolution of the sample components.

	COMPOUND	ION 300	ORH 801	COREGEL 64H	COREGEL 107H	COREGEL 87H3
1	Malic	12.0	7.5	11.5	10.2	10.0
2	Malonic	11.1	7.4	10.8	9.8	9.8
3	cis-Aconitic	7.5	7.4	11.2	9.9	9.6
4	Adipic	21.8	13.7	21.9	17.5	16.5
5	Formic	17.1	11.1	16.5	15.0	14.6
6	Maleic	8.1	5.2	7.7	7.6	7.8
7	Ascorbic	7.8	5.0	7.5	7.4	10.5
8	Butyric	—	18.5	—	24.1	—
9	Glycolic	15.4	9.9	14.8	13.4	12.8
10	Glycolic	11.5	7.4	11.1	10.1	9.8
12	Citric	9.5	6.3	9.1	8.4	8.3
13	Tartaric	10.0	6.4	9.6	8.9	8.7
14	Nicotinic	—	—	—	—	26.4
15	Propionic	20.1	15.2	21.7	19.3	18.6
16	Succinic	9.2	9.8	8.8	12.7	8.7
18	Oxalic	6.6	4.5	6.5	6.7	6.9
19	Sorbic	18.7	12.0	17.9	16.0	15.3
23	Acrylic	23.3	—	22.5	19.9	19.4
26	Isobutyric	—	—	—	21.8	21.4
27	Lactic	16.0	10.3	15.5	13.6	13.1
28	Shikimic	15.5	9.7	14.7	12.5	12.1
29	Fumaric	16.4	10.3	15.7	14.0	13.1
30	Glutaric	18.2	11.3	17.2	14.7	14.0
31	Pyruvic	9.3	6.0	9.0	8.6	8.7
32	Acetic	—	12.4	18.4	16.6	—
33	Proponal	28.7	28.9	—	—	—
36	Quinic	12.6	7.9	12.1	10.7	10.4

Test Conditions: 2.5 mM H_2SO_4, 50 ºC, Flow Rate 0.5 mL/minute, 100 ppm

The chart below illustrates the variety of columns available from Concise Separations compared to similar columns offered by other companies:

CONCISE SEPARATIONS	6% XL	6.4% XL	7% XL	8% XL	10% XL	PARTICLE SIZE (uM)
Coregel ARH 601	X					6.5
Coregel 107H					X	8
Coregel 64H		X				10
Coregel 87H1				X		9
Coregel 87H3				X		9
Coregel ION 300	X					7
Coregel ION 310				X		8
Coregel ORH 801			X			9
Coregel USP L17				X		8
Coregel WA1				X		9
Bio-Rad						
Fast Analysis Column				X		9
Fermentation Monitoring				X		9
Aminex™ HPX-87H				X		9
Phenomenex						
Rezex™ RFQ-Fast Acid				X		8
Rezex™ RHM-Monosaccharide				X		8
Rezex™ ROA-Organic Acid				X		8
Shodex						
KC811				N/A		6
Sugar SH1011				N/A		6
Sugar SH1821				N/A		6
Supelco						
SUPELCOGEL™ HPN 59304-U				N/A		9
SUPELCOGEL™ 6-10H				N/A		9
SUPELCOGEL™ HPN 59346				N/A		9

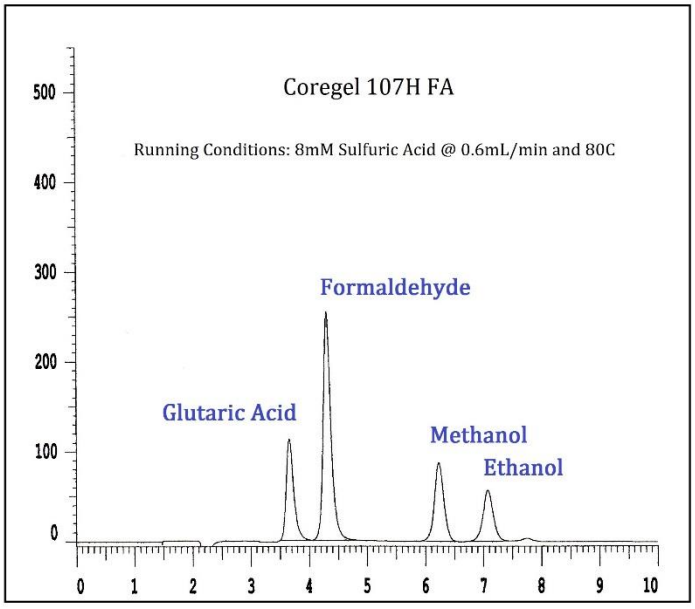
Bio Fuel Analysis

Concise Separations has worked with many Bio Fuel companies to develop Organic Acids columns that better fit their needs. The **107H FA** and the **801 FA** are the latest columns developed out of necessity to stream line important QC steps before and after fermentation.

Coregel 107H FA

PN: ICE-99-9867

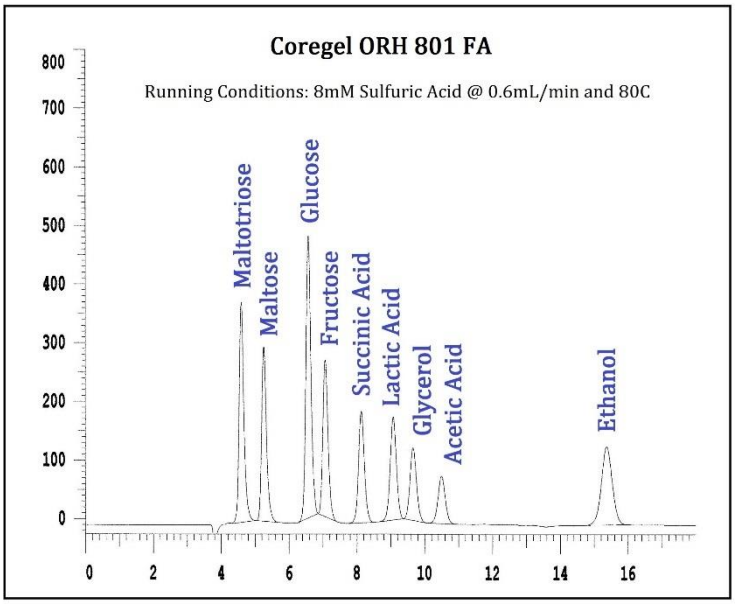
The **107H** polymer is unique to Concise Separations. The resolving power coupled with ruggedness allows the polymer to separate organic acids, aldehydes and alcohols in extreme environments. We created the **107H FA** for those Bio Fuel assays that need quick, Reliable results. Ethanol elutes near 7 minutes on the **107H FA** compared to 20 minutes on the original **107H** column.



Bio Fuel Analysis

Concise Separations developed another unique column targeted to more complicated Bio Fuel Analysis.

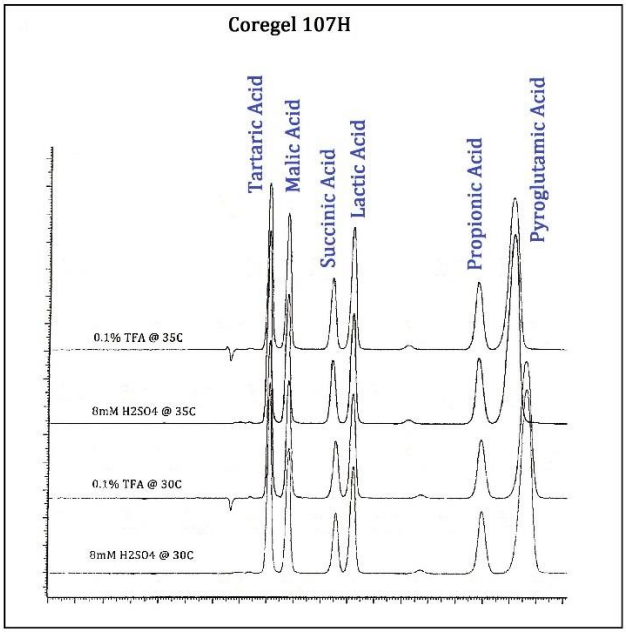
Coregel 801FA
PN: ICE-99-9753



The **801FA** results are much faster than the traditional **ORH 801** column yet peak efficiencies are not compromised.

Organic Acids with Mass Spectrometry

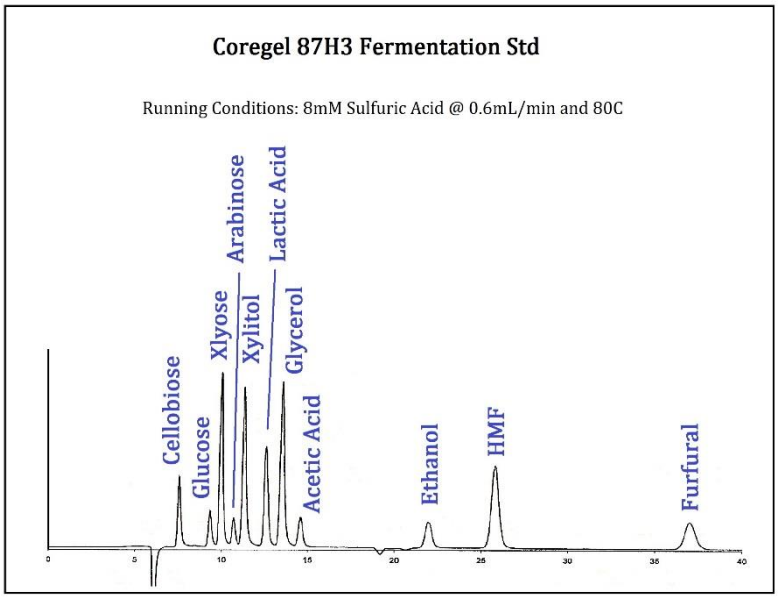
Trifluoroacetic Acid is shown to replace Sulfuric Acid as the eluent. This more volatile acid is favorable for MS Detection.



Fermentation Analysis

Concise Separations offers a variety of columns to best fit your fermentation analysis needs. The most popular is shown below.

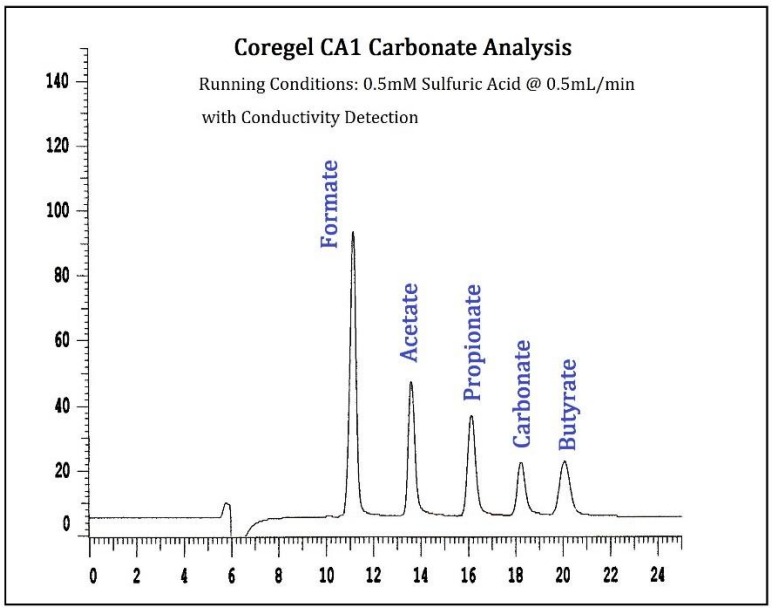
Coregel 87H3
PN: ICE-99-9861



Organic Acid Analysis with Conductivity

Have Conductivity Detector instead of UV? No worries. Concise Separations has a powerful column for you.

Coregel CA1 Carbonate Analysis Column
PN: ICE-99-9872



Tips on Maintaining the Performance of Coregel Columns

The most important fact to remember when using Coregel columns is that the polystyrene divinylbenzene copolymer is a low cross-linked material. This polymeric packing has a limited resistance to flow rate and pressure, and will irreversibly compact and overpressure at a certain level. Unlike polymers, silica based materials are not flow rate sensitive and the relation between pressure and flow rate remains relatively constant. Therefore, the Coregel columns should be carefully monitored for pressure, and should be operated within the recommended flow rates and pressure specifications.

- **Use column ovens to serve the dual purpose of increasing column efficiency and lowering column back pressure.**
- **Set the pressure shut off for the analytical test system at or slightly below the recommended pressure maximum for the column to prevent irreversible damage to the column.**
- **When installing, allow the column to warm up in the column oven for 15 minutes, and then start the flow rate below your target flow rate. After 15 minutes, increase the flow rate to the target flow rate and confirm that the column is operating at the expected back pressure.**
- **To increase the lifetime of your analytical column, we recommend the proper use of guard columns or cartridges. How frequently you change your guard column depends on pretreatment of the sample.**
- **Filter and remove potentially harmful organics from the samples to decrease the need to change guard columns. Carefully monitor the guard columns for pressure increase and the chromatograms for changes in retention and efficiency to determine the approximate useful lifetime of the guard columns.**

Customer Support

Please do not hesitate to contact us with any question about our products, or help with your application needs. By collaborating with customers to solve new sample separations, we not only provide solutions to problems for our valued customers, we discover new column methods and technologies that can benefit others in our industry. We encourage you to visit our website periodically for updates on new products and applications. We are continuously upgrading the website to provide easier access to specific applications and increase our valuable support information. Please feel free to contact us for website suggestions. Your opinion matters!

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