APPLICATION NOTE

CONCISE SEPARATIONS

ORGANIC ACID ANALYSIS HPLC COLUMNS

For over 30 years, Concise Separations has supplied the world with superior HPLC products and columns, providing customers with complete solutions for their separation needs. Our Organic Acids Analysis HPLC columns were developed specifically for analysis of fermentation products in the biofuels and food and beverage industries to separate sugars, sugar alcohols, acids and alcohols in the same run.

Ion exclusion is the preferred method for separating weakly ionizable species such as organic acids and alcohols. Concise Separations supplies a broad range of columns of varying efficiency and selectivity 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.

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.

In this bulletin, we highlight some new methods developed for customers using our Organic Acids Analysis HPLC columns.

// FEATURES & BENEFITS //

- Packed with chemically resistant polymeric polystyrene divinylbenzene copolymers varying in percent cross-linkage and particle sizes
- Stable in the pH range of 0 to 14
- Stable at high temperatures, up to 90 °C
- Consistent performance through numerous sample injections (dependent on sample preparation, instrument maintenance, and use of guard systems)
- Allows the use of universal detectors, such as refractive index (RI) indicators, due to the use of simple dilute acid, which doesn't require gradients for sample analysis
- Eliminates the need for high-cost solvents (including waste disposal)
- Eluent serves as a self-regenerating cleaning solution and does not degrade the column

// DONNAN MEMBRANE EXAMPLE EFFECT //

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

CHROMATOGRAPHY
SEPARATIONS
REPRODUCIBILITY
PRECISION

CPS analitica | Calibre SCIENTIFIC

Choosing a column for your sample

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. (See Figure 1 for an example) However, the many combinations of polymer cross-linkage, particle size, and column size offered by Concise Separations allow customers the freedom to choose a column with features most important to their separation needs.

Retention charts of common organic acids are another valuable tool when selecting a column. Due to the abundance of organic acids, sugars, and alcolhols, and the innumerable possible combinations of compouds in mixtures, it is impossible to create a comprehensive retention chart to guide   chemist through all applications. We stock most of the common organic acids, sugars, and alcohols in our laboratory, so we will frequently generate a sample chromatogram of your mixture or actually run your sample, to ensure our columns will separate your sample properly. Please contact Concise Separations with any questions regarding column selection.

// COLUMN CONSIDERATIONS //

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

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.

Although the 8% cross-linked polymeric columns provide excellent separation for many typical samples containing organic acids, sugars, and alcohols, Concise Separations has determined that certain separations are enhanced tremendously by varying cross-linkage and particle sizes of polymers. Although ion-exclusion of organic acids is the main separation mechanism for samples, the polymers' size exclusion limit is controlled by the percent cross-linkage. The additional size-exclusion mechanism for Concise Separations columns provides 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 speed, selectivity, and resolution of sample components in your separations.

Table 1 offers general guidelines for choosing your Concise Separations column. Our columns are listed and ranked according to their resolution quality, analysis time speed, selectivity, and durability.

Table 1

Resolution (Highest to Lowest)	Analysis Time (Fastest to Slowest)	Selectivity (Highest to Lowest)	Durability (Most to least)
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

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 optimal results, and to minimize the pressure limitations commonly found with other polymeric packings. By manipulating temperature and eluent strength, an analyst can greatly enhance species separation (see Figure 1). Unlike other column manufacturers, Concise Separations also offers different cross-linked organic acid columns to ultimately give the chromatographer control to optimize separations.

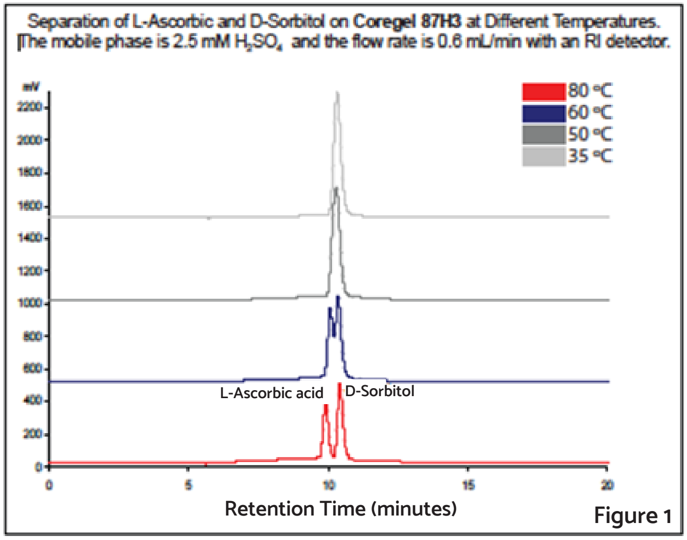


Figure 1

Biofuel Analysis

Concise Separations has worked with many biofuel companies to develop organic acids columns that better fit their needs. The 107H FA and the 801 FA are the latest columns developed to allow streamlining of important QC steps before and after fermentation.

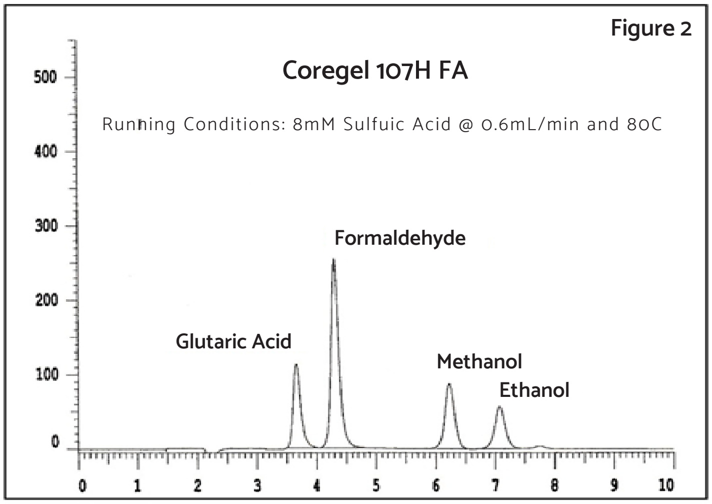


Figure 2

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 biofuel assays that need quick, reliable results. Ethanol elutes near 7 minutes on the 107H FA (see Figure 2) compared to 20 minutes on the original 107H column.

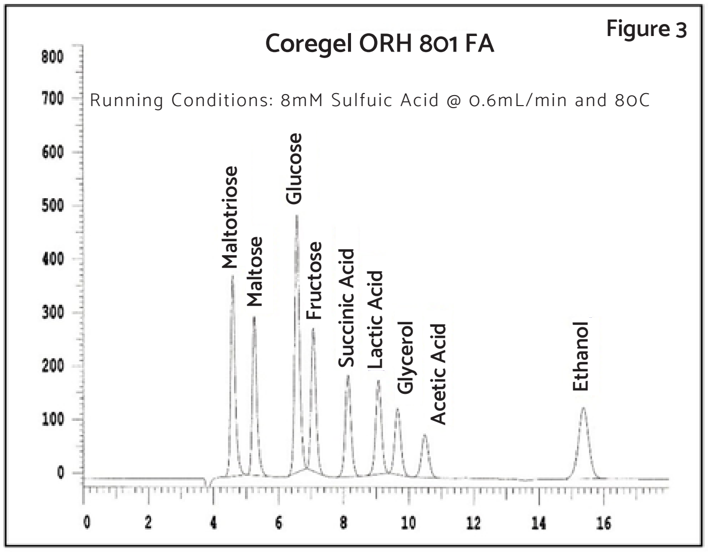
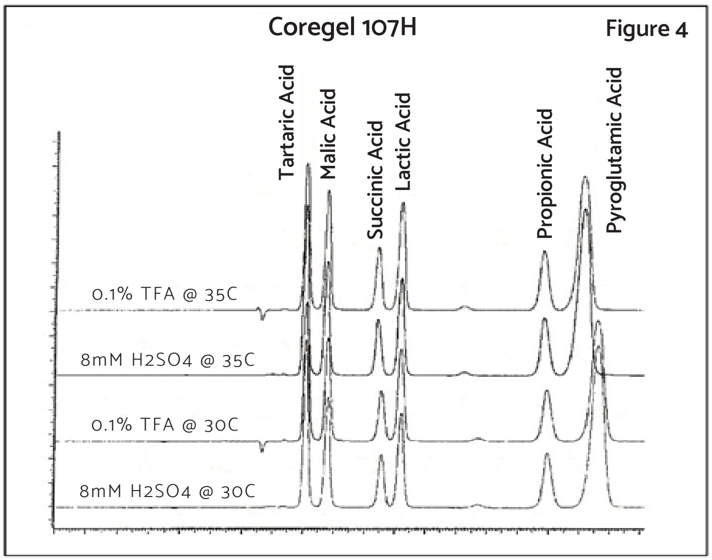


Figure 3

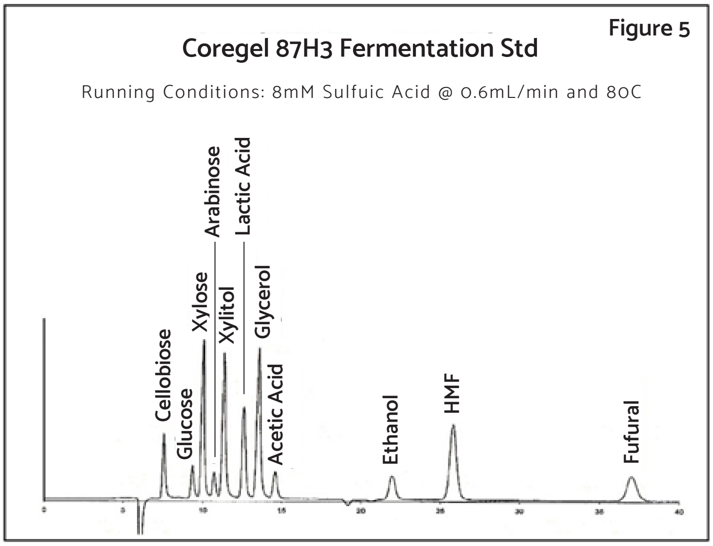
Coregel 801FA
PN: ICE-99-9753

For more complicated biofuel analysis, Concise Separations has developed the 801FA column: the 801FA results are much faster than the traditional ORH 801 column yet peak efficiencies are not compromised (see Figure 3).



Organic Acids with Mass Spectrometry

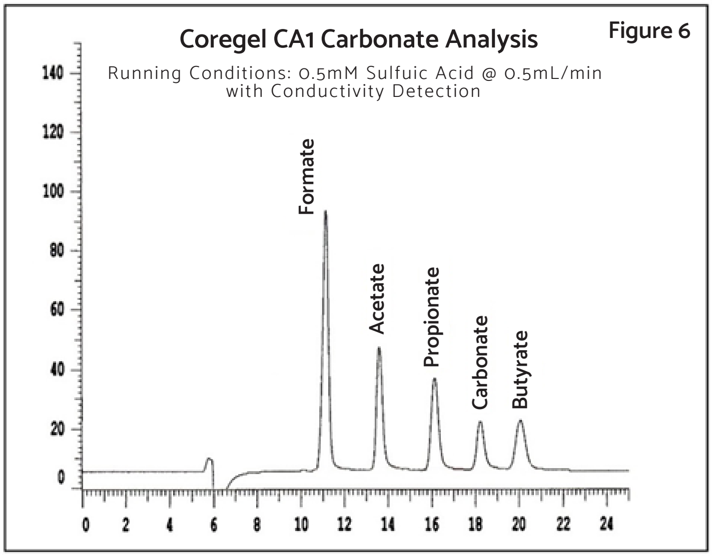
Mass spectrometry can be used for detection as well. For more favorable results from MS Detection, trifluoroacetic acid (TFA) replaces sulfuric acid (H_2SO_4) as the eluent, while giving the same retentions.



Fermentation Analysis

Concise Separations offers a variety of columns to best fit your fermentation analysis needs. Our most popular option, the Coregel 87H3, is shown in Figure 5.

Coregel 87H3
PN: ICE-99-9861



Organic Acid Analysis with Conductivity

Concise Separations has also developed a column for those who have conductivity detection instead of UV, the Coregel CA1 (see Figure 6).

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

The following table compares organic acid retention time between different cross-linked columns. The data for Table 2 was taken under the following test conditions: 2.5mM H_2SO_4, 50°C, Flow Rate 0.5 mL/minute, 100ppm.

Table 2

	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
17	Oxalic	6.6	4.5	6.5	6.7	6.9
18	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

Concise Separations offers a variety of organic acid columns to optimize your assays. Table 3 compares our columns to similar columns offered by other companies.

Table 3

	6% XL	6.4% XL	7% XL	8% XL	10% XL	Particle Size (µM)
Concise Separations						
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

Tips on Maintaining the Performance of CARBOSep Columns

The most important fact to remember when using CARBOSep 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, CARBOSep columns should be carefully monitored for pressure, and operated within the recommended flow rates and pressure specifications.

Customer Support

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

About Concise Separations

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.

// ADDITIONAL TIPS //

- Use column ovens to increase column efficiency and lower column back pressure.
- Set the pressure shut off for the analytical test system at or slightly below the recommended pressure maximum for the column used, to prevent irreversible damage to the column.
- When installing, allow the column to warm up in the column oven for 15 minutes, then start the flow rate below your target flow rate. After another 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 or purity of the sample.
- Filter and remove potentially harmful organics from samples to decrease the need to change guard columns. Carefully monitor the guard columns for pressure increase and monitor the chromatograms for changes in retention and efficiency to determine the approximate useful lifetime of the guard columns.

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