



## Chromatography Application Notes Organic Acids Analysis HPLC Columns

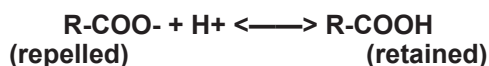
For over 25 years, Transgenomic, Inc. has supplied HPLC products to provide customers with solutions for their separation needs. We specialize in polymeric technologies used in a wide variety of HPLC columns, solid phase extraction products, analytical guard columns and cartridges, guard discs, and bulk polymers for purification and sample preparation applications. By providing consistent reliability and timely delivery of high quality, long-lasting products, we have established the Transgenomic 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

#### ICSep Columns for Organic Acid Analysis

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

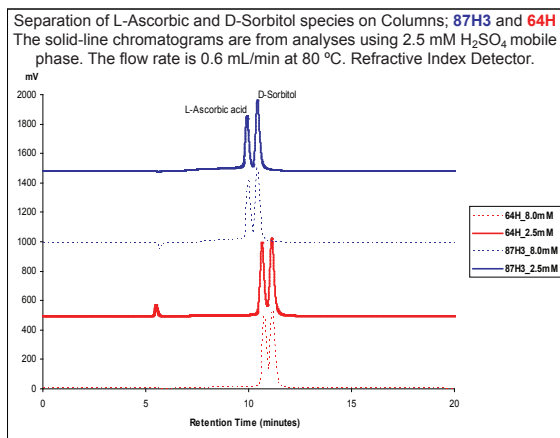
The polymeric packings employed with ion exclusion are totally sulfonated polystyrene-divinylbenzene (PS/DVB) copolymers. By totally sulfonating the polymer, the bead behaves as though it is a negatively charged sphere. This charged sphere is referred to as a Donnan membrane. Species that have a negative 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.



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.

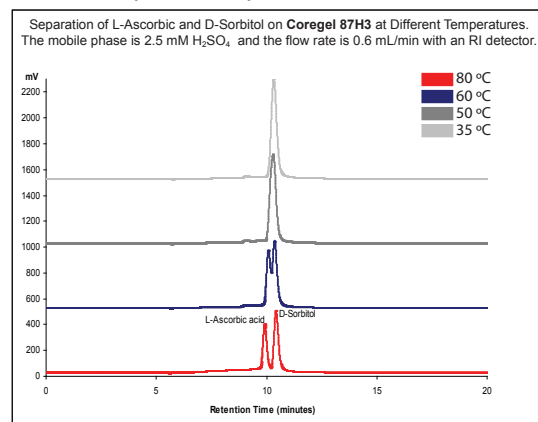
#### Eluent Effect

By controlling the strength of the acidic eluent, the retention times of the compounds can be influenced. The stronger (more acidic) the eluent, the longer the retention times in relation to the pKa. The eluent strength can be used by the analyst to enhance the separation of compounds:



#### Temperature Effect

By far the most powerful tool used to influence relative retention of compounds on Transgenomic 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

**ICSep** columns are packed with chemically resistant polymeric polystyrene-divinylbenzene copolymers varying in percent cross-linkage and particle sizes.

**ICSep** 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 °C
- 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 Transgenomic 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 Transgenomic allow customers to choose a column with features most important to maximize their separation needs.

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

Resolution (Highest to Lowest)	Analysis Time (Faster to Slower)	Selectivity (Higher to Lower)	Durability (More to Less)
ICSep ION300	ICSep USP L-17	ICSep ION300	ICSep Coregel 107H
ICSep Coregel 64H	ICSep Coregel 87H1	ICSep Coregel 64H	ICSep Coregel 87H3
ICSep WA-1	ICSep ION310	ICSep ORH801	ICSep Coregel 87H1
ICSep Coregel 87H3	ICSep ARH601	ICSep WA-1	ICSep WA-1
ICSep ORH801	ICSep ORH801	ICSep Coregel 87H3	ICSep ORH801
ICSep Coregel 107H	ICSep Coregel 107H	ICSep Coregel 107H	ICSep ION310
ICSep ARH601	ICSep Coregel 87H3	ICSep ARH601	ICSep USP L-17
ICSep ION310	ICSep WA-1	ICSep ION310	ICSep ARH601
ICSep Coregel 87H1	ICSep Coregel 64H	ICSep Coregel 87H1	ICSep Coregel 64H
ICSep USP L-17	ICSep ION300	ICSep USP L-17	ICSep ION300

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 Transgenomic for any questions regarding column selection.

	Compound	ION300	ORH801	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	Glycoctic	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<sub>2</sub>SO<sub>4</sub>, 50 °C, Flow Rate 0.5 mL/minute, 100 ppm

## Transgenomic Offers More Column Options

Transgenomic 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 co-polymer. Although the 8% cross-linked polymeric columns provide an excellent separation of many typical samples containing organic acids, sugars and alcohols, Transgenomic 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 Transgenomic columns provides unique selectivity for some components. For maximum efficiency, Transgenomic also provides columns with smaller particle sizes and tighter size distributions than the typical industry standard of 9 micron particles. By providing additional choices, Transgenomic allows you to maximize separations in terms of speed, selectivity and resolution of the sample components.

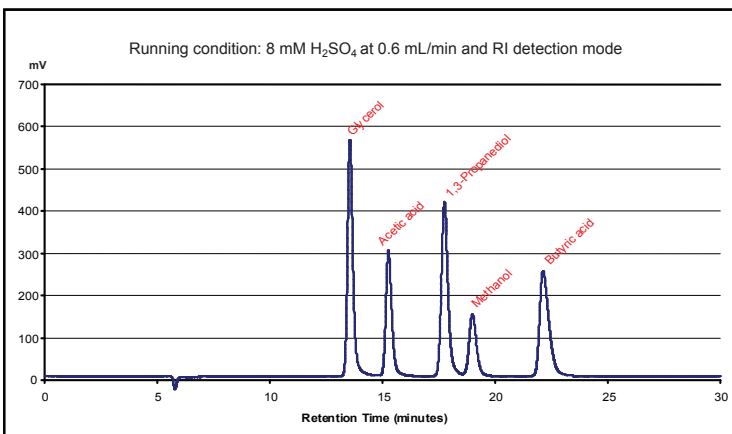
The chart below illustrates the variety of columns available from Transgenomic compared to similar columns offered by other companies:

Transgenomic	6% xl	6.4% xl	7% xl	8% xl	10% xl	Particle Size (µm)
ICSep ARH601	x					6.5
ICSep Coregel 107H					x	8
ICSep Coregel 64H		x				10
ICSep Coregel 87H1				x		9
ICSep Coregel 87H3				x		9
ICSep ION300	x					7
ICSep ION310				x		8
ICSep ORH801			x			9
ICSep USP L-17				x		8
ICSep WA-1				x		9
<b>Bio-Rad</b>						
Fast Analysis Column				x		9
Fermentation Monitoring				x		9
Aminex HPX-87H				x		9
<b>Phenomenex</b>						
Rezex RFQ-Fast Acid				x		8
Rezex RHM-Monosaccharide				x		8
Rezex ROA-Organic Acid				x		8
<b>Shodex</b>						
KC811				N/A		6
Sugar SH1011				N/A		6
Sugar SH1821				N/A		6
<b>Supelco</b>						
SUPELCOGEL™ HPN 59304-U				N/A		9
SUPELCOGEL™ 6-10H				N/A		9
SUPELCOGEL™ HPN 59346				N/A		9

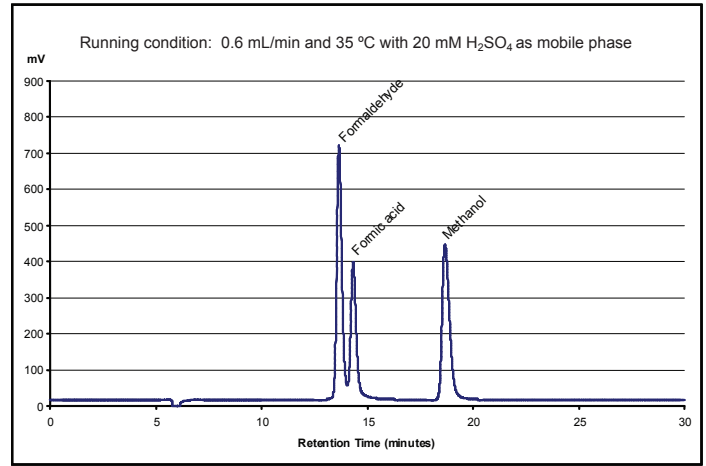
## New Applications

The following are new methods developed using our ICSep columns based on customer application requests.

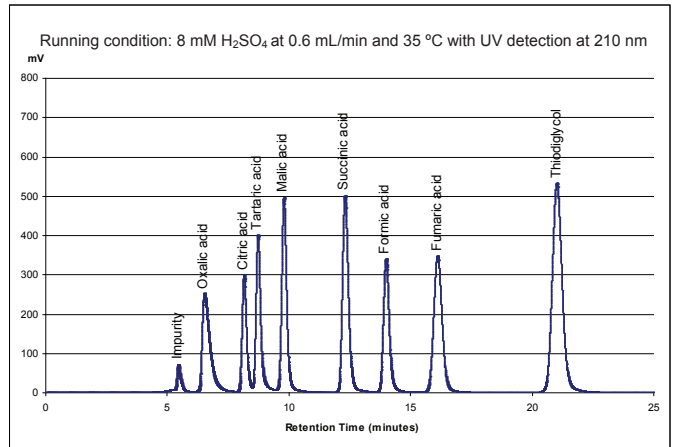
### Analysis of 1,3 – propanediol in the presence of other organic acids and methanol on the Coregel 87H3 column



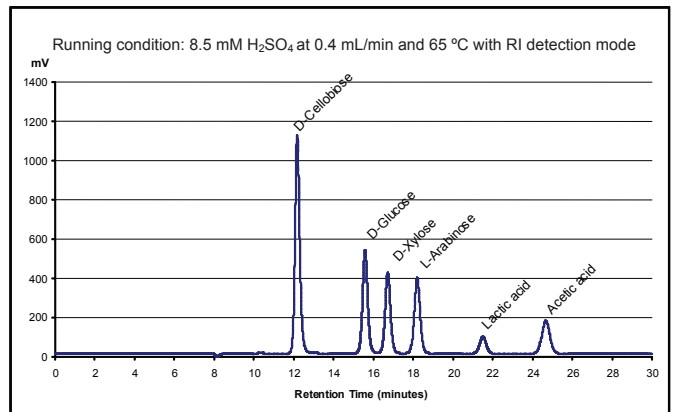
### Separation of formaldehyde in the presence of methanol and formic acid on the Coregel 87H3 column. UV detection at 210 nm.



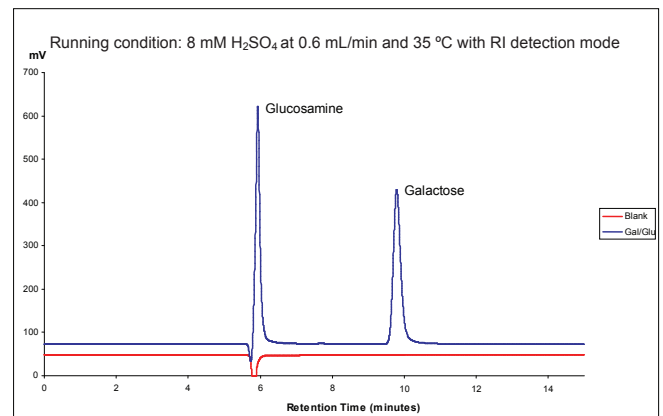
### Analysis of thiodiglycol Coregel 87H3 column



### Analysis of four sugars in the presence of two organic acids with the ION300 column



### Separation of galactose and the aminosugar glucosamine with the Coregel 87H3 column



## Tips on Maintaining the Performance of ICSep Columns

The most important fact to remember when using **ICSep** 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 **ICSep** 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.

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 compounds, and increase our valuable support information. Many of the website upgrades are in response to requests from our customers, so please keep sending in your helpful suggestions!

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