Essential Guide for Biofuel Analysis

Advanced Technologies

Tips

Troubleshooting

phenomenex®

...breaking with tradition℠




In the past few years, biofuel use and production has spread to every corner of the globe. As concerns over fossil fuel supplies and global warming increase, more nations are looking to renewable fuels in an effort to end oil dependency and decrease our negative environmental impacts. As the world's biofuel production and consumption rapidly increases, it is essential for manufacturers to have the latest cutting edge technologies at their disposal to meet this ever increasing demand.

Phenomenex is dedicated to supporting the biofuel industry with the most advanced chromatographic tools available for biofuel analysis. In this guide, we feature products designed to enhance production, simplify the biofuel analysis, and lower production costs. These technologies are changing the face of the biofuel industry and the way we look at alternative fuels today.

Biofuel Production Process

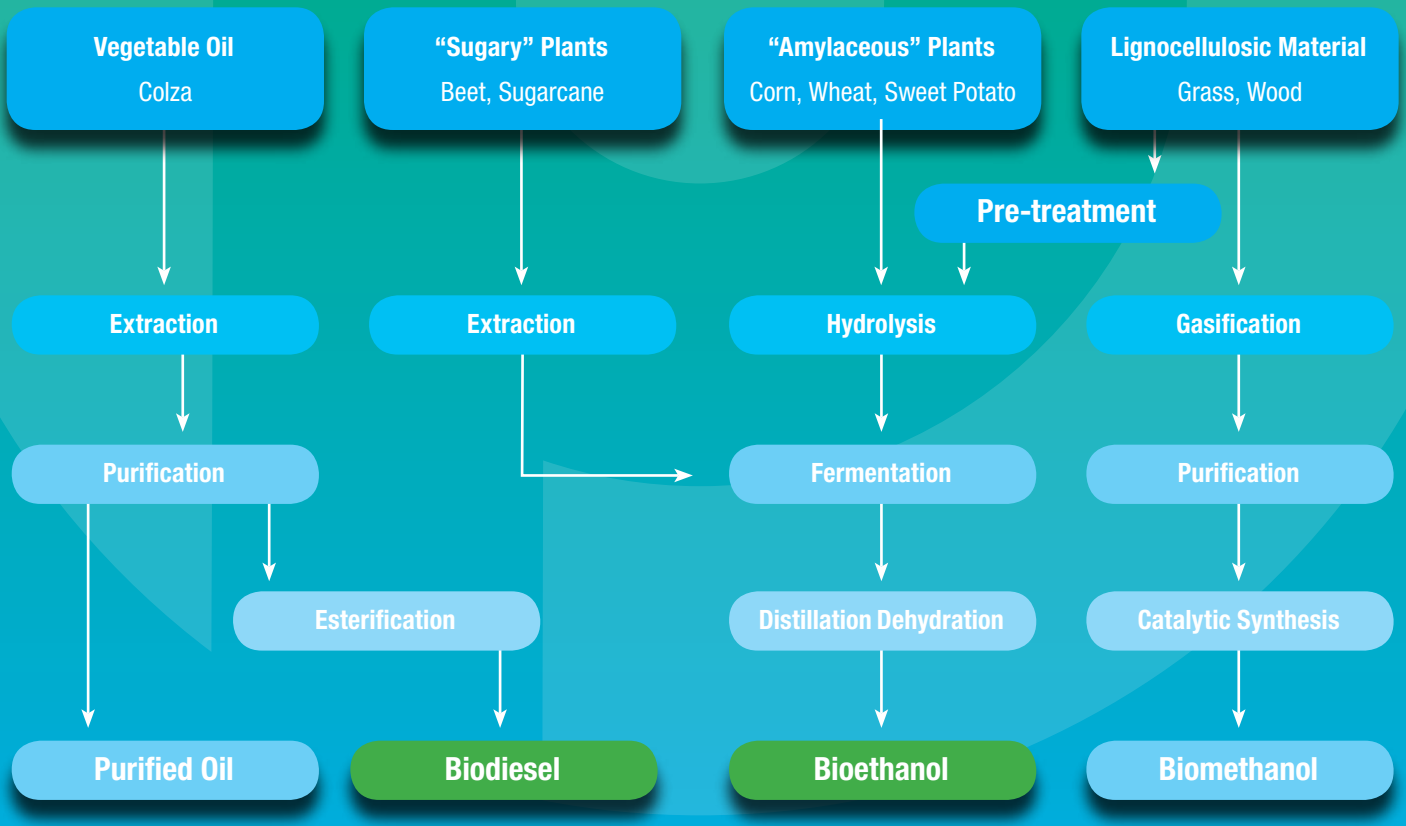


Table of Contents

guarantee

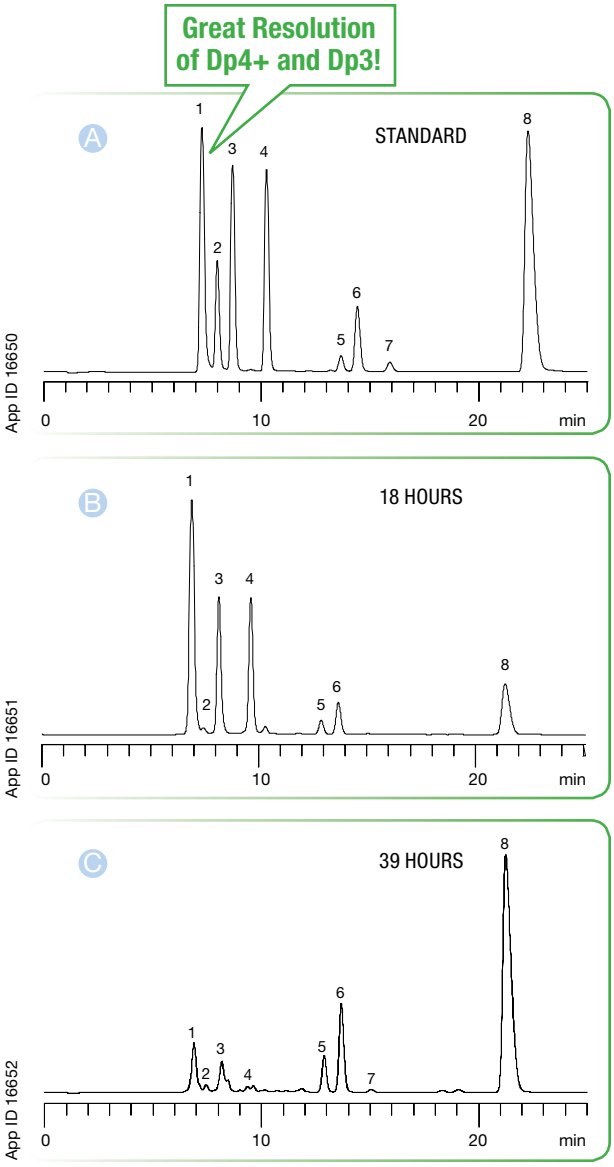
If you are not completely satisfied with any of the Phenomenex products featured in this brochure, return within 45 days for a FULL REFUND.*

*This guarantee is not valid in India and China.

Dependable Fermentation Monitoring with Rezex™ ROA HPLC Columns

- Easy quantitation of ethanol fermentation broth components
- Monitors starches, sugars, organic acids, and ethanol in one run
- Reliable lactic acid and acetic acid monitoring

Monitoring the key reaction components throughout the fermentation process is crucial for maximizing ethanol recovery. Rezex ROA is uniquely suited for the separation and analysis of simple and complex sugars, organic acids, and ethanol within a fermentation broth sample. With results easily obtained through an isocratic run, Rezex ROA is instrumental in helping you to accurately determine what critical steps need to be taken to ensure the maximum yield is achieved during your fermentation run.



Different fermentation runs on a Rezex ROA 300 x 7.8 mm column
(A) Bioethanol fermentation standard HPLC run. Note the excellent separation of all the components of interest. (B) Fermentation sample at 18 hours. (C) Fermentation sample at 39 hours. Note the depletion in the early oligosaccharide peaks and the increase in the ethanol peak as the fermentation progresses.

Increase Peak Sharpness and Separation

Did you know that a ThermaSphere™ TS-130 column heater serves many purposes? Not only does it reduce system backpressure, it also increases efficiency, which improves peak sharpness, lending to better baseline separation.

Easily Incorporate a Rapid and Effective Clean Up

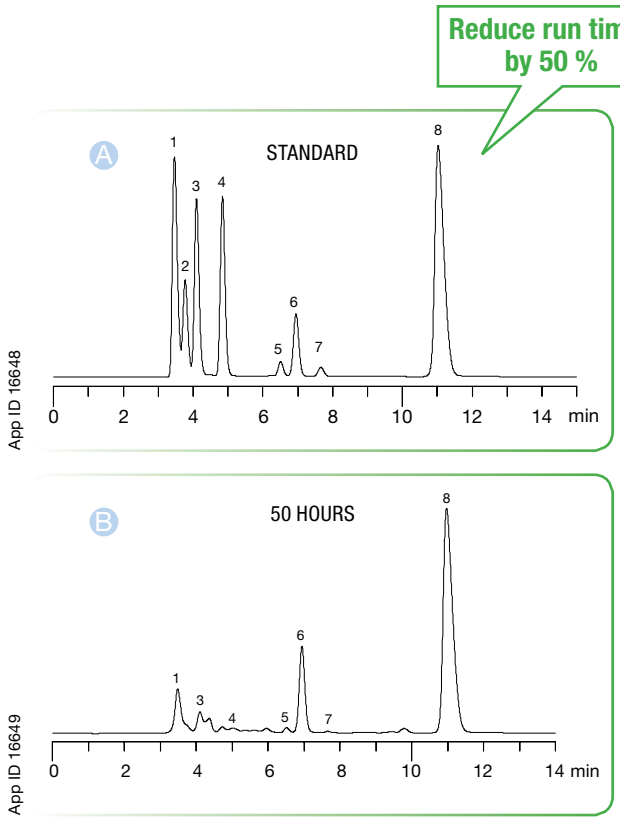
Filtration of fermentation samples with a SCX sample preparation tube (Strata® SCX) and a 0.20 or 0.45 µm syringe tip filter (Phenex™-RC) can reduce chromatographic interferences due to particulates and cationic impurities. This will increase run-to-run reproducibility and extend the lifetime of both the guard and analytical column.

Column:	Rezex ROA-Organic Acid
Dimensions:	300 x 7.8 mm
Part No.:	00H-0138-K0
Mobile Phase:	0.005 N Sulfuric Acid
Flow Rate:	0.6 mL/min
Detection:	RI @ 40 °C
Temperature:	60 °C
System:	Shimadzu™ Prominence™ LC-20A System
Sample:	1. Dp4+ 5. Lactic Acid
	2. Dp3 6. Glycerol
	3. Maltose 7. Acetic Acid
	4. Glucose 8. Ethanol

FAST Fermentation Analysis with Rezex™ ROA HPLC Columns

- Increase throughput by reducing run times 50 %
- Faster results that allow for more real-time responses
- Lower cost from decreased solvent usage

Rezex ROA has the ability to achieve excellent base line separation between Dp3 and Dp4+, which have proven to be a challenge within the bioethanol industry. It is this great base line separation that affords scientists the opportunity to utilize a shorter column dimension. By using the 150 x 7.8 mm Rezex ROA column, you are able to decrease the run time by 50 % when compared to the average run time on a 300 x 7.8 mm column.



Different fermentation runs on a Rezex ROA 150 x 7.8 mm column
(A) Bioethanol fermentation standard HPLC run. (B) Late fermentation time point run. Note that the low level of remaining oligosaccharide peaks allows for some quantitation. The late eluting ethanol and organic acid peaks are well resolved on the shorter column with reduced run time as compared to the Rezex ROA 300 x 7.8 mm column, shown on the previous page.

Extend Column Lifetime

Protect the HPLC column from the intrusion of the metal ions by using SecurityGuard™. The SecurityGuard guard cartridge system works by trapping metal ions, such as calcium, magnesium, and iron, which can damage the column and cause it to lose or change separation efficiency.

Knowledgeable Customer Service Available

Don't get put on hold – get answers when you need them. Phenomenex has experienced biofuel specialists available to answer your questions. Email: ***info@phenomenex.com*** for a biofuel specialist.

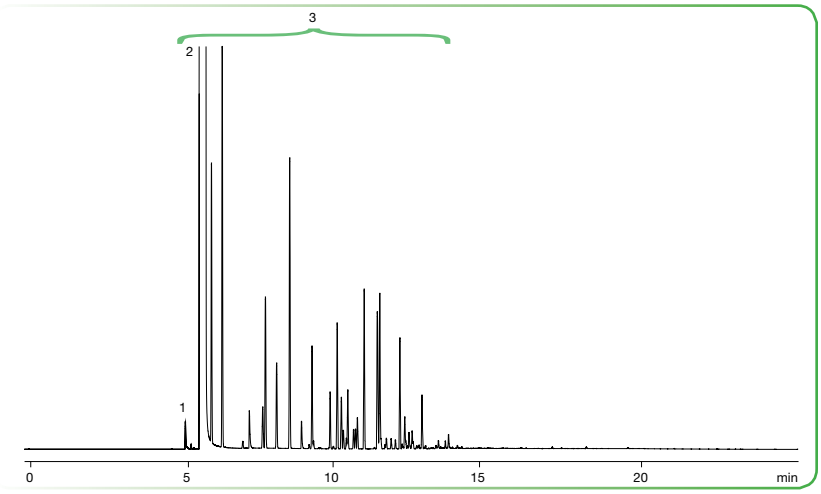
Column:	Rezex ROA-Organic Acid
Dimensions:	150 x 7.8 mm
Part No.:	00F-0138-K0
Mobile Phase:	0.005 N Sulfuric Acid
Flow Rate:	0.6 mL/min
Detection:	RI @ 40 °C
Temperature:	60 °C
System:	Shimadzu™ Prominence™ LC-20A System
Sample:	1. Dp4+ 5. Lactic Acid 2. Dp3 6. Glycerol 3. Maltose 7. Acetic Acid 4. Glucose 8. Ethanol

Accurate Ethanol Measurements with Zebron™ ZB-1 GC Columns

- Complete resolution of methanol and ethanol from all denaturant components
- More accurate analysis with extremely low bleed levels
- Reliable results guaranteed from strict quality assurance parameters

Determining the ethanol and methanol content of the final fuel product can be very challenging. The analysis requires a GC column capable of separating ethanol and methanol from all other components (such as fusel alcohols and denaturants).

The Zebron ZB-1 GC column is engineered under the strictest quality assurance parameters to ensure that you get the best separation possible. Its Engineered Self-Crosslinking™ (ESC) technology provides extremely low bleed levels for highly reproducible and reliable results.



App ID 16663

ASTM D 4806

ASTM D 5501

prEN 15376

ASTM Method D 5501: Determination of Denatured Bioethanol

Column:	Zebron ZB-1
Dimensions:	100 meter x 0.25 mm x 0.50 µm
Part No.:	7MG-G001-17
Injector:	Split 50:1 @ 300 °C, 1 µL
Carrier Gas:	Helium @ 35 cm/sec (Constant Flow)
Oven Program:	45 °C for 7 min to 255 °C @ 30 °C/min (Hold 6 min)
Detector:	FID @ 300 °C
Instrument:	Shimadzu™ GC-2010 with Flame Ionization Detection and AOC-20i Automatic Liquid
Sample:	1. Methanol 2. Ethanol 3. Denaturant

Accurate and Painless GC Column Installation

Did you know that many GC problems are related to improper column installation? To ensure the best chromatography, use the Cool-Lock™ Nut to get the proper installation depth. This patent pending tool will also help you to avoid burning your fingers while installing your column accurately in record breaking time. For more information, call your local Phenomenex specialist.

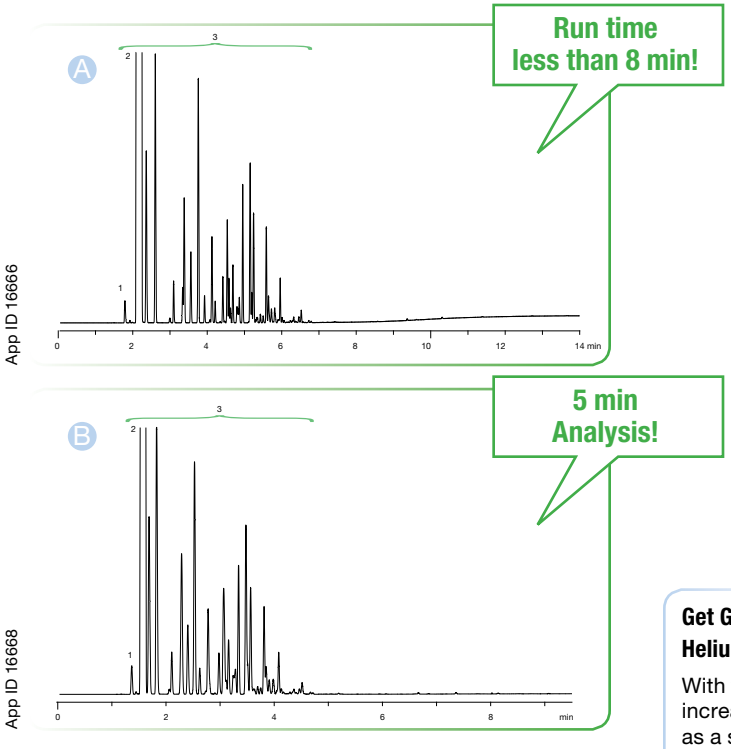
NEW

FAST Quality Checks with Zebron™ ZB-Bioethanol GC Columns

- Quick determination of ethanol content with shorter run times
- Complete resolution of methanol and ethanol peaks
- Complete your certificate of analysis in a fraction of the time

The standard GC method for bioethanol can take up to 40 minutes per sample. Though this long run time is often necessary to ensure the proper separation of methanol and ethanol from the rest of the fuel components, it slows down the quality assurance process and may delay fuel shipments. To save time, some plants ship their fuel supply before the certificate of analysis (COA) is completed and risk the chance of recalling their shipment if the quality assurance test fails.

To streamline the quality assurance process, Phenomenex developed the new Zebron ZB-Bioethanol GC column for fast and accurate analysis of methanol and ethanol in the final fuel product. The Zebron ZB-Bioethanol GC column utilizes a proprietary phase that is specially designed to give quick and accurate separation of methanol and ethanol.



ASTM D 4806
ASTM D 5501
prEN 15376

ASTM Method D 5501: Determination of Denatured Bioethanol

Column: Zebron ZB-Bioethanol
Dimensions: A) 30 meter x 0.25 mm x 1.00 µm
B) 15 meter x 0.25 mm x 1.00 µm
Part No.: A) 7HG-G020-22
B) 7EG-G020-22
Injector: Split 50:1 @ 300 °C, 1 µL
Carrier Gas: A) Hydrogen @ 35 cm/sec (Constant Flow)
B) Hydrogen @ 25 cm/sec (Constant Flow)
Oven Program: A) 45 °C for 2.5 min to 255 °C @ 30 °C/min (Hold 4.5 min)
B) 55 °C for 1.7 min to 260 °C @ 40 °C/min (Hold 2.67 min)
Detector: FID @ 300 °C
Instrument: Shimadzu™ GC-2010 with Flame Ionization Detection and AOC-20i Automatic Liquid
Sample: 1. Methanol
2. Ethanol
3. Denaturant

Get Great Chromatography with Hydrogen or Helium Gas

With the recent helium shortage and its rapid price increase, many labs are switching to hydrogen as a safe and cost-effective alternative. This change however, may affect factors, such as velocities and efficiencies. The ZB-Bioethanol column is designed to give fast and reliable results regardless of the carrier gas used. Please contact your local GC specialist for more information.

Get a FREE Biofuel Technical Note
Visit: www.Phenomenex.com/info/biofuels
Enter Code: 5748

Reliable Determination of Glycerin in Biodiesel with Zebron™ ZB-5HT Inferno™ GC Columns

2007 Top 100 Technologies

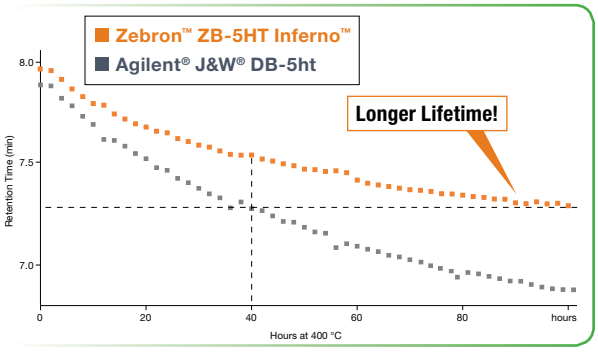
- Long column lifetime with 430 °C temperature limits
- Avoid column breakage and brittleness
- Eliminate the need for metal columns

To ensure the quality of the biodiesel product, many methods specify a high temperature analysis of free and total glycerin. However at temperatures above 380 °C, most fused silica columns become brittle and spontaneously break.

Phenomenex has a unique fused silica column designed specifically for high temperature biodiesel analysis. This award winning column, the Zebron ZB-5HT Inferno™, is specially processed to be thermally stable up to 430 °C. Its stationary phase and polyimide coating is more rugged and can withstand higher temperatures than other conventional columns, making the Zebron ZB-5HT Inferno ideal for biodiesel analysis.

Zebron ZB-5HT Inferno™ Outperforms Other Fused Silica Columns

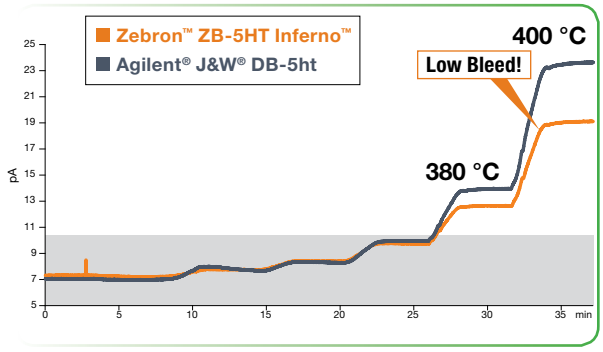
Studies have found that Phenomenex's Zebron ZB-5HT is well suited for high temperature analyses. When compared to Agilent's DB-5ht, the Zebron ZB-5HT has less bleed, more thermal stability, and longer lifetime.



Zebron ZB-5HT outperforms Agilent J&W DB-5ht. Columns were held at 400 °C for 2 hours, then the oven was lowered to 120 °C for Pentadecane analysis.

Conditions for all columns:

Dimensions: 30 meter x 0.25 mm x 0.10 µm
Injection: 1.0 µL
Carrier Gas: Helium @ 1.9 mL/min (Constant Flow)
Oven Program: 120 °C Isothermal
Detector: FID @ 400 °C
Sample: Pentadecane



Column bleed profile comparison of an Agilent® J&W DB-5ht column versus a Zebron ZB-5HT Inferno column.

Conditions for all columns:

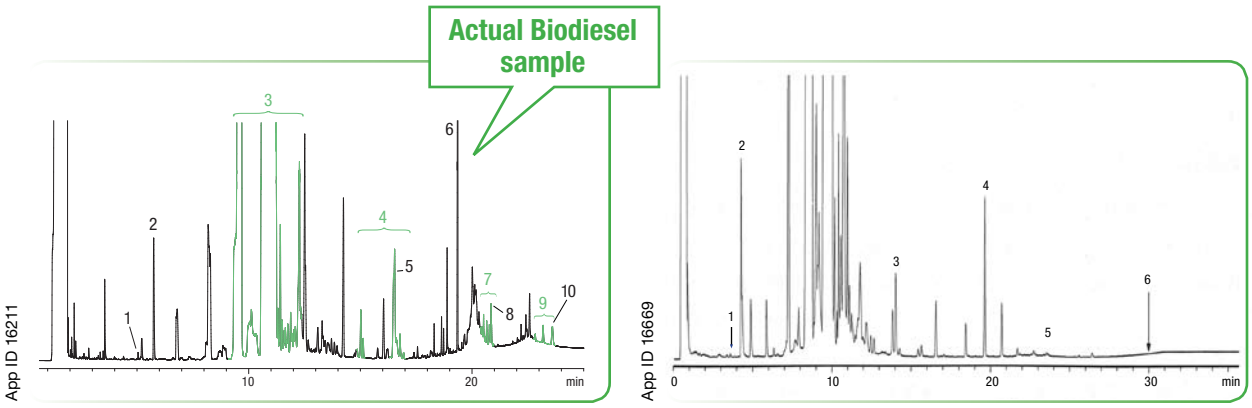
Dimensions: 30 meter x 0.25 mm x 0.10 µm
Injection: Null Injection @ 250 °C
Carrier Gas: Hydrogen @ 11 psi (Constant Pressure)
Oven Program: 120 °C for 3 min to 320 °C @ 30 °C/min (hold 5 min) to 340 °C @ 30 °C/min (hold 5 min) to 360 °C @ 30 °C/min (hold 5 min) to 380 °C @ 30 °C/min (hold 5 min) to 400 °C @ 30 °C/min (hold 5 min)
Detector: FID @ 405 °C

The shaded area depicts the bleed criteria for MS certified columns on a MS detector. MS bleed certification values are typically read at 320 °C. This demonstrates the low bleed capabilities of the ZB-5HT. It meets MS certification limits even at 360 °C!

ASTM D 6584
EN 14105
EN 14106
ABNT NBR 15341
ABNT NBR 15344

All columns used for above tests were new/never used, prior to this testing and purchased either directly from the original manufacturer or through an authorized distributor. All testing was carefully controlled to ensure conditions were similar for all columns involved. The comparative data may not be representative of every application.

Reliable Determination of Glycerin in Biodiesel with Zebron™ ZB-5HT Inferno™ GC Columns *(cont'd)*



ASTM Method D 6584:
Free and Total Glycerin in B-100 Biodiesel Methyl Esters

Column: Zebron ZB-5HT Inferno
Dimensions: 15 meter x 0.32 mm x 0.10 µm
Part No.: 7EM-G015-02
Injection: On-Column @ 53 ºC, 1 µL
Carrier Gas: Helium @ 3.0 mL/min (constant flow)
Oven Program: 50 ºC for 1 min to 180 ºC @ 15 ºC/min to 230 °C @ 7 ºC/min to 380 ºC @ 30 ºC/min for 10 min
Detector: FID @ 380 ºC
Sample:
1. Glycerol
2. Butanetriol (ISTD1)
3. Esters
4. Monoglycerides
5. 1-Monooleoyl-rac-glycerol
6. Tricarpin (ISTD2)
7. Diglycerides
8. 1,3-Diolein
9. Triglycerides
10. Triolein

Note: A 2.0 m x 0.53 mm Guard Column was connected to the analytical column per ASTM method requirements.

EN 14105:
Free and Total Glycerin Analysis in Biodiesel

Column: Zebron ZB-5HT Inferno
Dimensions: 10 meter x 0.32 mm x 0.10 µm
Part No.: 7CM-G015-02
Injection: On-Column, 1 µL
Carrier Gas: Helium @ 120 cm/sec
Oven Program: 50 °C for 1 min to 180 °C @ 15 °C/min to 230 °C at 7 °C/min to 370 °C at 10 °C/min (hold 5 min)
Detector: FID @ 380 °C
Sample:
1. Glycerol
2. Butanetriol (ISTD1)
3. Monoolein
4. Tricarpin (ISTD2)
5. Diolein
6. Triolein

Note: A 5 m x 0.53 mm Guard Column was connected to the analytical column.

Ensure Reproducible and Reliable Glycerin Analysis

Phenomenex is committed to providing you with the highest quality products to ensure the most accurate and reliable glycerol analysis. Every column is individually QC tested to give excellent batch-to-batch reproducibility and the highest performance possible.

ASTM D 6584
EN 14105
EN 14106
ABNT NBR 15341
ABNT NBR 15344

ORDERING INFORMATION

Bioethanol Columns

Rezex™ ROA-Organic Acid Columns

HPLC column for bioethanol anlaysis

Part No.	Dimensions	Unit	Price
00H-0138-K0	300 x 7.8 mm	ea	
00F-0138-K0	150 x 7.8 mm	ea	
00G-0138-E0	250 x 4.6 mm	ea	
03B-0138-K0	50 x 7.8 mm, Guard Column	ea	

Zebron™ GC Columns

Gas chromatography columns ideal for ethanol quality checks

Part No.	Description	Unit	Price
7HG-G020-22	Zebron ZB-Bioethanol, 30 meter x 0.25 mm x 1.00 µm	ea	
7EG-G020-22	Zebron ZB-Bioethanol, 15 meter x 0.25 mm x 1.00 µm	ea	
7MG-G001-17	Zebron ZB-1, 100 meter x 0.25 mm x 0.50 µm	ea	
AG0-5155	Zebron ZB-1 Test Mix	ea	

Note: Use SecurityGuard to extend column lifetime. See HPLC Accessories for more information.

Biodiesel Columns

Zebron™ Inferno™ GC Columns

Gas chromatography columns ideal for biodiesel analysis

Part No.	Description	Unit	Price
7EM-G015-02	Zebron ZB-5HT, 15 meter x 0.32 mm x 0.10 µm	ea	
7EM-G015-02-GST	Zebron ZB-5HT, 15 meter x 0.32 mm x 0.10 µm, + 2 meter x 0.53 mm Spliced Guard	ea	
7CM-G015-02	Zebron ZB-5HT, 10 meter x 0.32 mm x 0.10 µm	ea	
7CM-G015-02-GST	Zebron ZB-5HT, 10 meter x 0.32 mm x 0.10 µm, + 2 meter x 0.53 Spliced Guard	ea	
AG0-5155	Zebron ZB-1HT & ZB-5HT Inferno Test Mix	ea	

Sample Preparation

Strata® SCX

Sample preparation tubes for quick and easy clean up

Part No.	Description	Unit	Price
8B-S010-EAK	Strata SCX Tube, 55 µm, 70 Å, 100 mg/1 mL	100/Box	

Phenex™ Syringe Filters

Rapid filtration of samples prior to analysis

Part No.	Description	Membrane Type/Size	Unit	Price
AF0-3203-12	4 mm Diameter for < 2 mL Sample Volumes	0.20 µm Phenex-RC (Regenerated Cellulose)	100/pk	
AF0-3203-52	4 mm Diameter for < 2 mL Sample Volumes	0.20 µm Phenex-RC (Regenerated Cellulose)	500/pk	
AF0-3103-12	4 mm Diameter for < 2 mL Sample Volumes	0.45 µm Phenex-RC (Regenerated Cellulose)	100/pk	
AF0-3103-52	4 mm Diameter for < 2 mL Sample Volumes	0.45 µm Phenex-RC (Regenerated Cellulose)	500/pk	

HPLC Accessories

SecurityGuard™

Universal Guard Cartridge System

Part No.	Description	Price
KJ0-4282	SecurityGuard Guard Cartridge Kit	
AJ0-4490	SecurityGuard Cartridges, Carbo-H$^+$, 4 x 3.0 mm, 8/pk	

ThermaSphere™ TS-130 Column Heater

Improves reproducibility, peak efficiency, analyte quantitation, and chromatographic results

Part No.	Description	Price
EH0-7057	ThermaSphere TS-130 HPLC Column Heater 25-90 °C, 95 to 265 VAC, 50/60 Hz	
EH0-7058	Stand for ThermaSphere TS-130 HPLC Column Heater	

ORDERING INFORMATION

GC Accessories

Cool-Lock™ Nut*

GC nut for accurate and precise column installation

Part No.	Description	Unit	Price
AG0-8319	Cool-Lock GC Capillary Nut For Use With Short-Style Ferrules	ea	
AG0-8320	Cool-Lock GC Capillary Nut For Use With Long-Style Ferrules	ea	
AG0-8349	Cool-Lock Nut Installation Gauge	ea	
Replacement			
AG0-4701	GC Capillary Ferrules Graphite 1/16 in. to 0.5 mm ID	10/pk	
AG0-4704	GC Capillary Ferrules Graphite 1/16 in. to 0.8 mm ID	10/pk	

** For Agilent GC Systems*

guarantee

If you are not completely satisfied with any of the Phenomenex products featured in this brochure, return within 45 days for a FULL REFUND.*

*This guarantee is not valid in India and China.

Inlet Base Seals*

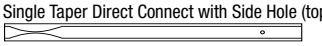
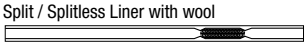
Seals liner with injection port for better peak shape and quantitation

Part No.	Description	Similar to Manufacturer Number **	Unit	Price
Standard, single groove for splitless applications, 0.8 mm dia. inlet hole				
AG0-7518	Gold Inlet Base Seal, splitless (single groove), 0.8 mm	18740-20885	2/pk	
AG0-7519	Gold Inlet Base Seal, splitless (single groove), 0.8 mm	18740-20885	10/pk	
High Split Flow, cross groove for split applications, 0.8 mm dia. inlet hole				
AG0-7520	Gold Inlet Base Seal, split (double groove/cross), 0.8 mm	5182-9652	2/pk	
AG0-7521	Gold Inlet Base Seal, split (double groove/cross), 0.8 mm	5182-9652	10/pk	
Replacement Inlet Seal Washers				
AG0-7522	Inlet Seal Washers, for Agilent GC injection port	5061-5869	12/pk	

** For Agilent GC Systems. ** Similar to but not always an exact equivalent to the original manufacturer's product.*

Injection Port Inlet Liners

Focuses and concentrates sample to improve reproducibility and results

Description	GC Model No.	Dimensions ID x L x OD (mm)	Material* (deactivated)	Quartz Wool (Y/N)	Similar to Manufacturer Number **	Part No.	Unit	Price
Agilent Technologies (HP)								
Single Taper Direct Connect with Side Hole (top) 	5880/5980/6890	4 x 78.5 x 6.3	B (y)	N	21055	AG0-7850	5/pk	
Single Taper Direct Connect with Side Hole (bottom) 	5880/5980/6890	4 x 78.5 x 6.3	B (y)	N	G1544-80730 20771	AG0-7851	5/pk	
Split / Splitless Liner with wool 	5880/5890/6890	4 x 78.5 x 6.3	B (y)	Y	—	AG0-8174	5/pk	
Shimadzu™								
Split / Splitless Liner 	9A/16A	3.4 x 139 x 5.0	B (y)	N	20749	AG0-4669	5/pk	
Thermo Scientific (Finnigan)								
Splitless / Single Taper Liner Trace / Trace 	8000	5 x 105 x 8.0	B (y)	N	45350033	AG0-7852	5/pk	

** B= Borosilicate; Deactivated = Yes (y) or No (n). ** Similar to but not always an exact equivalent to the original manufacturer's product.*

Long Ferrules

Seals injection inlet to syringe to reduce coring and increase septum lifetime

Part No.	Description		Unit	Price
0.4 mm Ferrule ID				
AG0-4698	Graphite Ferrule	1/16 in. to 0.4 mm	10/pk	
AG0-4699	Graphite Ferrule	1/16 in. to 0.4 mm	50/pk	
0.5 mm Ferrule ID				
AG0-4701	Graphite Ferrule	1/16 in. to 0.5 mm	10/pk	
AG0-4702	Graphite Ferrule	1/16 in. to 0.5 mm	50/pk	
0.8 mm Ferrule ID				
AG0-4704	Graphite Ferrule	1/16 in. to 0.8 mm	10/pk	
AG0-4705	Graphite Ferrule	1/16 in. to 0.8 mm	50/pk	

Septa

Enhanced durability and re-sealing capabilities

Part No.	Description	Unit	Price
PhenoRed 3/8 in. (9.5 mm) Diameter 400 °C			
AG0-4690	PhenoRed-400 Injector Septa	50/pk	
AG0-4691	PhenoRed-400 Injector Septa	100/pk	
PhenoRed 7/16 in. (11 mm) Diameter 400 °C			
AG0-4696	PhenoRed-400 Injector Septa	50/pk	
AG0-4697	PhenoRed-400 Injector Septa	100/pk	

Essential Guide for Biofuel Analysis

Advanced Technologies

Tips

Troubleshooting

www.phenomenex.com
Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department by telephone, fax or email: international@phenomenex.com.

phenomenex®
...breaking with tradition℠

	Australia	Austria	Canada	Denmark	France	Germany
mail:	PO Box 4084 Lane Cove, NSW 2066 Australia	Zeppelinstr. 5 63741 Aschaffenburg Germany	411 Madrid Ave. Torrance, CA 90501-1430 USA	Gydevang 39-41 3450 Allerød Denmark	Parc des Grillons, Bat.3 60 route de Sartrouville 78232 Le Pecq Cedex France	Zeppelinstr. 5 63741 Aschaffenburg Germany
tel.:	02-9428-6444	01-319-1301	(800) 543-3681	4824 8048	01 30 09 21 10	06021-58830-0
fax:	02-9428-6445	01-319-1300	(310) 328-7768	4810 6265	01 30 09 21 11	06021-58830-11
email:	auinfo@ phenomenex.com	anfrage@ phenomenex.com	info@ phenomenex.com	dkinfo@ phenomenex.com	franceinfo@ phenomenex.com	anfrage@ phenomenex.com

	Ireland	Italy	New Zealand	Puerto Rico	United Kingdom	USA
mail:	Queens Avenue, Hurdsfield Ind. Est., Macclesfield, Cheshire SK10 2BN, UK	Via Serenari, 15/D 40013 Castel Maggiore (BO) Italy	P O Box 31-601 Milford 0741 North Shore City New Zealand	273 Sierra Morena, Suite #104 San Juan, Puerto Rico 00926	Queens Avenue, Hurdsfield Ind. Est., Macclesfield, Cheshire SK10 2BN, UK	411 Madrid Ave. Torrance, CA 90501-1430 USA
tel.:	01 247 5405	051 6327511	09-4780951	(800) 541-HPLC	01625-501367	(310) 212-0555
fax:	+44 1625-501796	051 6327555	09-4780952	(310) 328-7768	01625-501796	(310) 328-7768
email:	eireinfo@ phenomenex.com	italiainfo@ phenomenex.com	nzinfo@ phenomenex.com	info@ phenomenex.com	ukinfo@ phenomenex.com	info@ phenomenex.com

5748_I