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# Bioalcohol and Biodiesel Application Notebook

Choice of complete solutions – timely critical analysis

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# Introduction

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### Biofuel production. The Global Challenge.

Biofuel is defined as a solid, liquid, or gas fuel derived from biological material. This broad-based class of biofuel compounds can be separated into two categories:

- Bioalcohol
- Biodiesel

There are Thermo Scientific™ solutions for every step of your workflow process. Whether the solutions employ near-infrared (NIR) spectroscopy or ion (IC), liquid (LC), or gas chromatography (GC), we can deliver critical information about your biofuel process in a timely manner. We offer a choice of systems and models to best suit your specific application requirements and budget.



# BioFuel Production

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Biofuels are energy sources made from living things (plants and animals) or their byproducts. From research to production, the goal is to maximize the benefits and viability of these energy sources while minimizing any drawbacks.

**Bioalcohol** comes from crops such as corn, sugar cane, wheat, sorghum, and cellulosic plants such as corn stover, wood, and grasses. With the exception of sorghum, these crops are not naturally high in sugars. However, the grains are high in starch, and the rest of the plant is rich in cellulose and hemicellulose.

Making the cellulose more accessible to hydrolysis and solubilizing hemicellulosic sugars is currently difficult and expensive. The analytical challenge is quantifying the diverse mixture of sugars present in hemicellulose.

**Biodiesel** is a fatty acid methyl ester (FAME). A FAME is derived from vegetable or animal fats which have undergone a transesterification process with an alcohol, catalyzed by sodium or potassium hydroxide. Currently the vast majority of bio-refineries are using vegetable oils as feed stock for the production of biodiesel.

In biodiesel, the ability to quantify fatty acid methyl esters (FAMES) and trace contaminants is key to ensuring final product quality.



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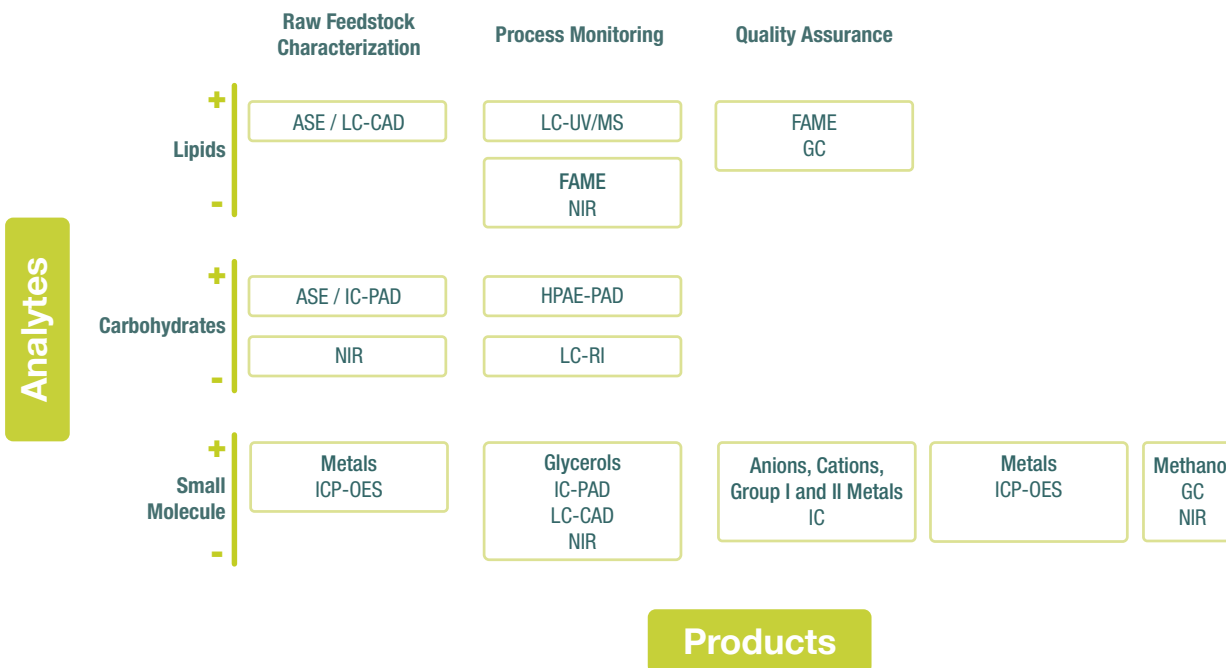
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Chromatography and spectroscopy solutions can deliver critical information about the biofuel process in a timely manner. The workflow positions a choice of available technologies according to analytes of interest and process steps. The technologies include accelerated solvent extraction (ASE), IC, high pressure LC (HPLC), GC, near infrared (NIR), and inductively coupled plasma optical emission spectroscopy (ICP-OES), which are ranked according to sensitivity and can be used in research, development or production environments.

[Download the Brochure BioFuel Workflows](#)

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# Analytical Technologies



## Chromatography

From sample preparation to final results, discover how our complete portfolio of chromatography products can improve your analytical performance and workflow productivity.

- Sample Preparation
- Ion Chromatography
- Liquid Chromatography
- Gas Chromatography

Learn more at [thermoscientific.com/chromatography](https://thermoscientific.com/chromatography)



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## Sample Preparation Accelerated Solvent Extraction

The Thermo Scientific™ Dionex™ [ASE™ 150 or 350](#) Accelerated Solvent Extraction system uses elevated temperatures and pressures to rapidly extract water- or oil-soluble components in cellulosic and algal biomass samples.



Accelerated Solvent Extraction  
Dionex ASE 150/350 System

Learn more at [thermoscientific.com/ase](http://thermoscientific.com/ase)

## Ion Chromatography Best-in-class resolution, speed, and sensitivity

The Thermo Scientific™ Dionex™ [ICS-5000+ HPIC™ system](#)—with the ability to operate continuously up to 5000 psi—provides fast, high-resolution ion chromatography analysis using the latest 4 µm columns.



ICS-5000+ HPIC System

Learn more at [thermoscientific.com/ic](http://thermoscientific.com/ic)



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## Liquid Chromatography

### Redefining High Pressure and Ultra High Pressure LC to give more

The Thermo Scientific™ Dionex™ [UltiMate™ 3000](#) liquid chromatography systems allow you to choose from a wide variety of modules and configurations to create an ultra high pressure LC (UHPLC) instrument configuration that is perfect for your application.



Ultimate 3000 LC System

Learn more at [thermoscientific.com/liquidchromatography](https://thermoscientific.com/liquidchromatography)

## Gas Chromatography

### The sensitivity and productivity needed in today's lab

The Thermo Scientific™ [TRACE™ 1300 Series](#) Gas Chromatograph is the latest technology breakthrough conceived to substantially elevate performance in QA/QC and routine laboratories.



TRACE 1300 Series GC

Learn more at [thermoscientific.com/en/products/gc-systems.html](https://thermoscientific.com/en/products/gc-systems.html)

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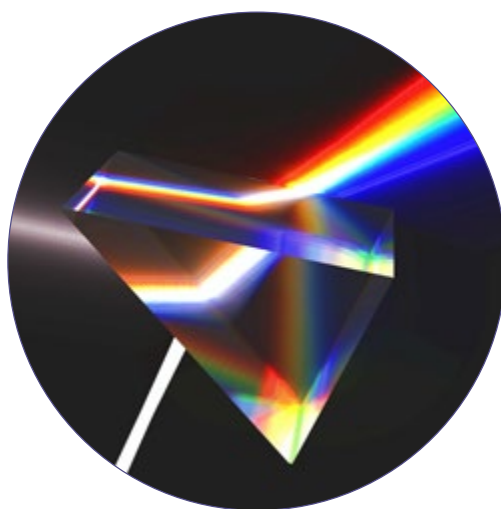
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# Analytical Technologies



## Spectroscopy

Meet the needs of your biofuels applications with our proven instruments for spectroscopy and elemental analysis. Have confidence and get definitive answers from smart systems that are big on performance.

- Inductively Coupled Plasma
- Near-Infrared Spectroscopy





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## Inductively Coupled Plasma Small in size, big on performance

The Thermo Scientific™ [iCAP™ 7000 Series](#) ICP-OES is ideal for direct oil analysis, providing high matrix tolerance and excellent sensitivity for raw material identification.



iCAP 7000 Series ICP-OES

Learn more at [thermoscientific.com/icp](https://thermoscientific.com/icp)

## Near-Infrared Spectroscopy Rapid, reliable measurements

The Thermo Scientific™ [Antaris™ II NIR analyzer](#) provides robust and reliable data collection for at-line, online, and in-line analysis. Analyze raw feedstock by reflection using the internal integrated sphere, liquids with the internal temperature-controlled transmission module, or process monitoring with fiber optics probes—all in one turnkey system.



Antaris II NIR Analyzer

Learn more at [thermoscientific.com/nir](https://thermoscientific.com/nir)



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Alcohol produced from biomass, called bioalcohol, is an important renewable energy source. As bioalcohol is produced from biomass by sugar fermentation, it is necessary to determine which types of plants produce the best yields of usable sugars.

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# Bioalcohol



## Raw Material Characterization

The quality and handling of raw materials is both critical and challenging in the successful output of bioalcohol. It is necessary to manage byproducts of the process as well as identify key inhibitors in addition to determining optimal sample concentrations and ideal method analysis.

- IC-PAD
- HPLC
- NIR



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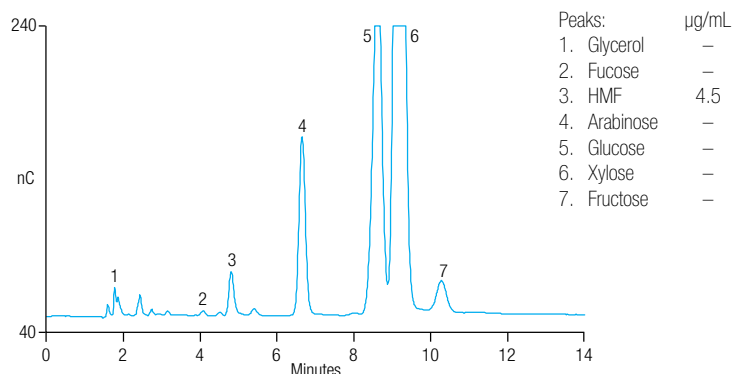
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## Raw Material Characterization Biomass Sugars and HMF Analysis using IC-PAD

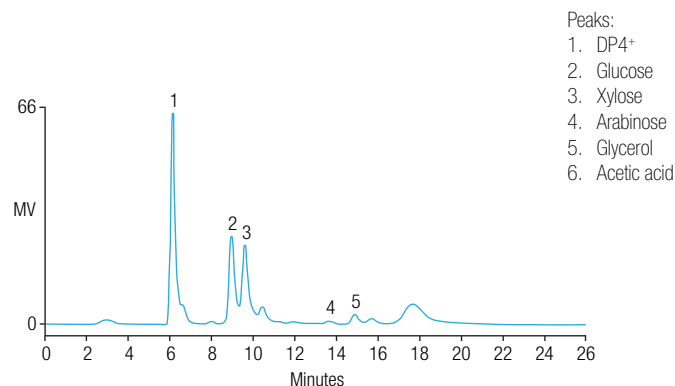


Here, hydroxymethylfurfural (HMF), a byproduct and inhibitor of ethanol processing, was detected in acid-hydrolyzed corn stover without interference from the other sugars using high-performance anion exchange chromatography with Pulsed Amperometric Detection (HPAE- PAD). Total run time to provide high sample throughput using a Thermo Scientific™ Dionex™ [CarboPac™ PA 1 Column](#) was 15 minutes, suggesting this method is ideal for on-line monitoring of HMF during biomass processing.

[Download Application Note 270  
Determination of Hydroxymethylfurfural in Honey and Biomass](#)

[Download Application Note 1044  
Determination of Anions in Dried Distillers Grains with Solubles](#)

## Raw Material Characterization Pre-hydrolyzed Switchgrass Analyzed by HPLC



Switchgrass samples were acid-hydrolyzed to determine the optimal concentration for recovery of glucose. Samples pretreated with 0.5% H<sub>2</sub>SO<sub>4</sub> showed a significant increase in glucose, as seen in the figure above. Samples pretreated with a lower concentration (0.2% H<sub>2</sub>SO<sub>4</sub>) showed xylose was the predominant species (results not shown).

[Download Application Note 363: Using Accelerated Solvent  
Extraction in Alternative Fuel Research](#)



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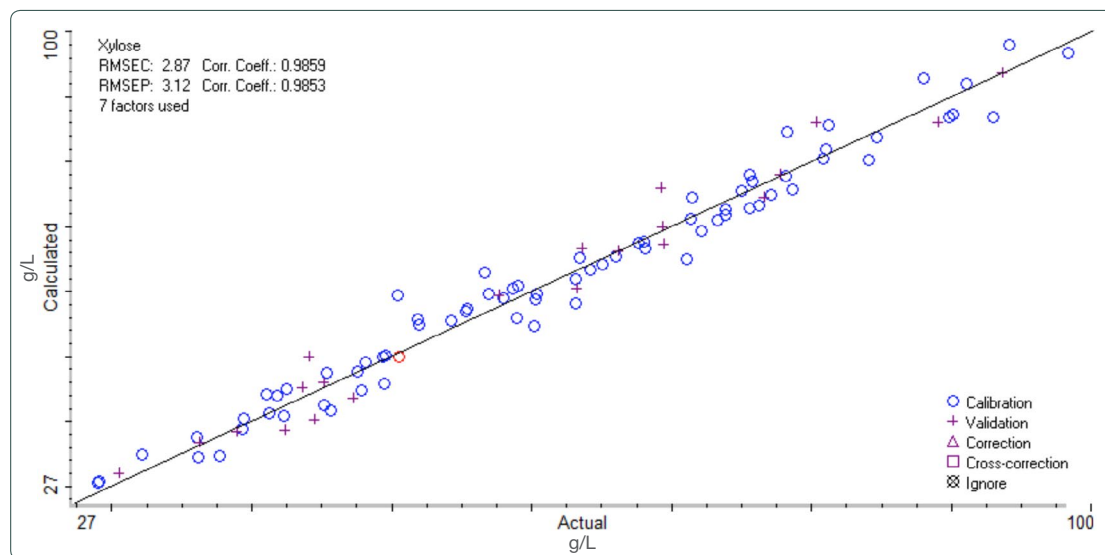
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## Raw Material Characterization Calibration Curve for Measurement of Xylose by NIR



In this slide, the carbohydrate xylose is analyzed using NIR. One of the distinguishing features of NIR is the absence of any peaks produced from the analysis. A sample is analyzed through comparison with partial least squares (PLS) calibration curves made from stock solutions across a given concentration range.

[NIR spectroscopy](#), while a relatively less sensitive technique than IC, HPLC, GC, and ICP, enables rapid analysis of critical components of process feedstock in a few seconds, without the use of consumables or reagents.

[View the Webinar: Near Infrared Analysis of Biofuels](#)

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## Process Monitoring

During the production process, key parameters and components must be carefully measured and monitored including bioalcohols and unwanted byproducts.

 HPLC-RI



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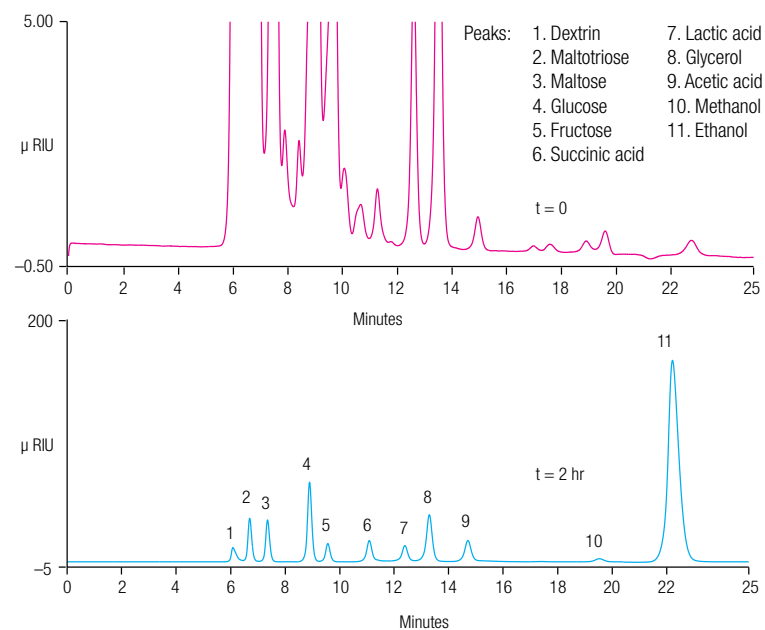
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## Process Monitoring Fermentation Analysis of Carbohydrates by HPLC-RI

During the fermentation process, three key parameters (including eight components) can be easily monitored and quantitatively analyzed by HPLC refractive index (HPLC-RI):

- 1) The amount of ethanol being produced is shown in the two chromatograms taken at two time points
- 2) At the start of the fermentation process, the amount of fermentable sugars (dextrin, maltotriose, maltose, and glucose) in the fermentation broth
- 3) Two hours later, the concentration of unwanted byproducts (lactic acid, acetic acid, and glycerol) that are produced



[Download Application Note 225: Rapid Method for the Estimation of Total Free Monosaccharide Content of Corn Stover Hydrolysate Using HPAE-PAD](#)

[Download Application Note 282: Rapid and Sensitive Determination of Biofuel Sugars by Ion Chromatography](#)

[Download Application Note 192: Carbohydrate Determination of Biofuel Samples](#)

[View the Webinar: Feedstock Characterization and Fermentation Monitoring of Biofuels](#)

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# Bioalcohol



## Quality Control

To ensure quality ethanol and butanol for fuel additives, it is necessary to quickly be able to measure total and potential sulfates and total chloride fast run times.

Elements occurring in the production process such as Copper, Sulfur, and Phosphorus must also be limited to ensure systems continue to run cleanly and smoothly without blockages or corrosion.

● IC

● ICP-OES





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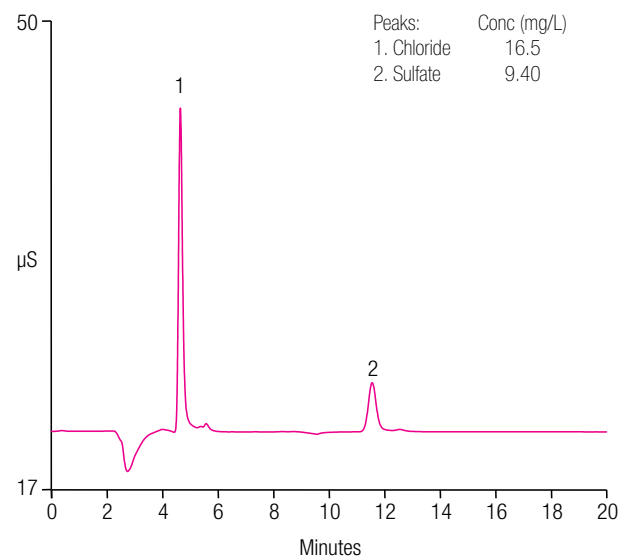
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## Quality Control Total & Potential Sulfate & Total Chloride in Butanol by IC

The results shown here are from a direct injection IC approach to determine total and potential sulfates and total chloride in butanol. Run time was under 15 minutes using a Thermo Scientific™ Dionex™ [IonPac™ AS22 Carbonate Eluent Anion-Exchange Column](#) and suppressed conductivity detection.



Download Application Note 296: Assay of Fuel-Grade Butanol for Total and Potential Sulfate and Total Chloride Per ASTM D7328-07

Download Application Note 297: Determination of Total and Potential Sulfate and Total Chloride in Fuel-Grade Butanol Per ASTM D7319-09

Download Application Note 1052: Determination of Chloride and Sulfate in Gasoline-Denatured Ethanol

Download Application Note 290: Determination of Total and Potential Sulfate and Total Chloride in Ethanol According to ASTM Method D7319



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## Quality Control Analysis of Cu, S, and P in Ethanol by ICP-OES

	Detection Limit mg/L	1 hour check		2 hour check		3 hour check		4 hour check	
		Measured mg/L	Recovery %	Measured mg/L	Recovery %	Measured mg/L	Recovery %	Measured mg/L	Recovery %
Cu 324.754 nm	0.0015	0.150	100.0 %	0.160	106.7 %	0.161	107.3 %	0.156	104.0 %
P 177.495 nm	0.011	0.701	93.5 %	0.709	101.3 %	0.713	95.1 %	0.741	98.8 %
S 180.731 nm	0.021	7.427	99.0 %	6.826	91.0 %	6.911	92.1 %	7.015	93.5 %

Results from stability tests that measured the levels of Cu, S, and P (elements that can result in engine gum deposits, formation of corrosive sulfur dioxide that can lead to acid, and poison the catalysts used in exhaust systems, respectively) were within acceptable limits with all of the recoveries with 10% of the prepared value.

Download Application Note 40971: Petrochemical Series – Accurate determination of copper, phosphorus and sulfur in ethanol using the Thermo Scientific iCAP 6000 Series ICP



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Biodiesel is made when vegetable oil or animal fats are chemically reacted with an alcohol resulting in fatty acid methyl or ethyl esters. It can be used as a cleaner-burning fuel in standard diesel engines, or blended with traditional petrodiesel creating additional fuel options with cleaner emissions.

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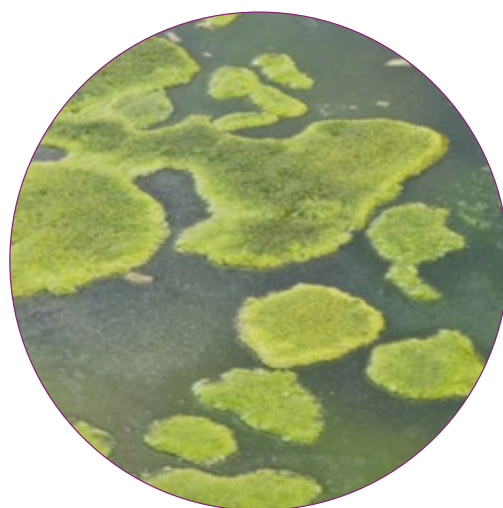
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## Raw Material Characterization

In the production of biodiesel fuels, raw materials are characterized to measure for many molecular species, to determine the most efficient methods of extraction, and to determine the elemental content of oils to determine the best candidates for producing biodiesel.

- HPLC-CAD
- ASE
- ICP-OES



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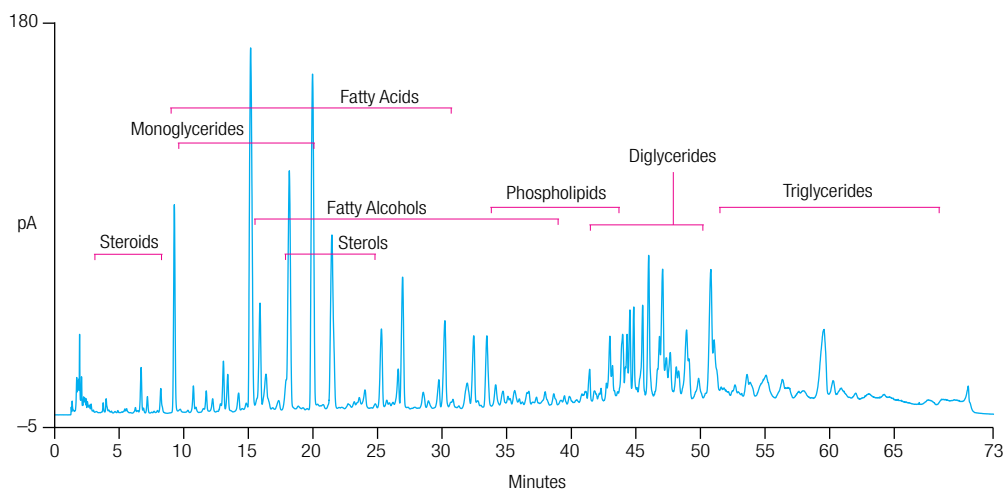
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## Raw Material Characterization Lipid Profiling of Algae Oil using HPLC-CAD



This reversed-phase analytical method effectively characterizes lipid samples obtained from algae oil extracts. [HPLC](#) with charged aerosol detection (CAD) has the sensitivity to detect low-level compounds for the researcher or analytical chemist, and has reduced chemical requirements (analytes are only required to be nonvolatile) to allow for a broad range of molecular species to be measured.

Download Application Note 71759: Analysis of Lipids  
by RP-HPLC Using the Corona ultra



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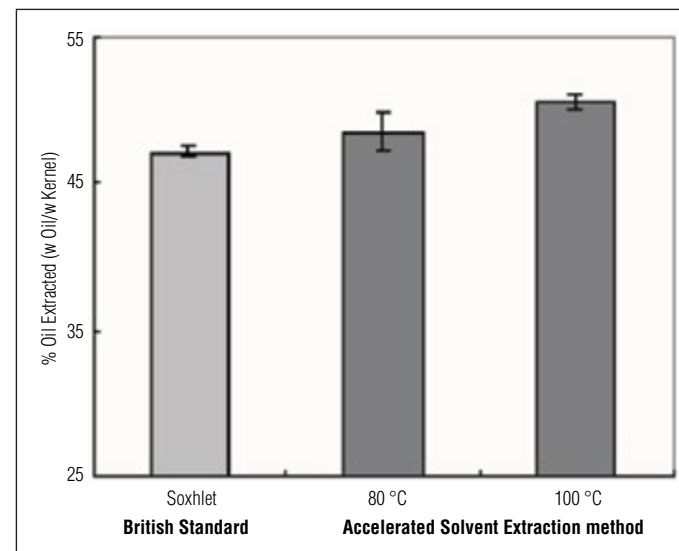
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## Raw Material Characterization Determination of Oil Content in Biodiesel Feedstock

The extraction efficiency of Soxhlet vs. [ASE](#) is compared using Jatropha seeds (a type of feedstock used in the production of biodiesel). Results from the gravimetric analysis of the extracted oil content using Soxhlet with hexane, refluxed at 68 °C provides comparable recovery to ASE with hexane at 80 °C and 100 °C, within experimental error. The advantage of using ASE is the extraction is completed in 21 minutes vs Soxhlet which takes 11 hours.



Download Customer Application Note 301:  
Determination of Oil Content in Biodiesel Feedstock  
by Accelerated Solvent Extraction



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### Elemental Analysis of Canola Oil by ICP-OES

	Crude	Refined	Bleached	Deodorized	Detection Limit
<b>Ca 317.9 nm</b>	162.1	1.6	0.22	0.05	0.0019
<b>Cu 324.7 nm</b>	0.036	<DL	<DL	<DL	0.0011
<b>Fe 259.9 nm</b>	1.17	<DL	<DL	<DL	0.00092
<b>Mg 280.2 nm</b>	61.57	0.611	0.07	0.006	0.00025
<b>Na 589.5 nm</b>	0.122	1.145	0.28	<DL	0.011
<b>Ni 231.6 nm</b>	<DL	<DL	<DL	<DL	0.0013
<b>P 177.4 nm</b>	282.109	3.018	1.213	0.579	0.0072
<b>Pb 220.3 nm</b>	<DL	<DL	<DL	<DL	0.010
<b>S 180.7 nm</b>	7.031	2.93	1.484	3.495	0.058
<b>Sn 189.9 nm</b>	0.112	0.075	0.089	0.01	0.0037

Determination of the elemental content of canola oil by [ICP](#) at four stages of the refining process. As expected, the concentration of the elements decreases with a higher degree of processing. The concentrations for calcium and magnesium are below the Association of Analytical Communities (AOAC) Official Method Ca 17-01 allowable threshold in the deodorized canola, making it a good candidate for producing either biodiesel or foodstuffs.

Download Application Note 40876: Elemental Analysis of Canola Oil Using the Thermo Scientific iCAP 6500 ICP

Download Brochure 70701: Edible Oil Workflows Compositional and Trace Contaminant Analysis

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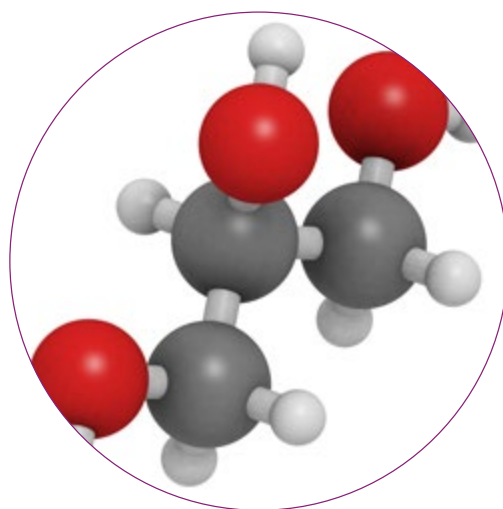
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## Process Monitoring

Monitoring the biodiesel production process requires the careful determination of glycerols, lipids, and other key substances such as alcohols and carbohydrates throughout the process.

- GC-FID
- HPLC-CAD
- IC-PAD





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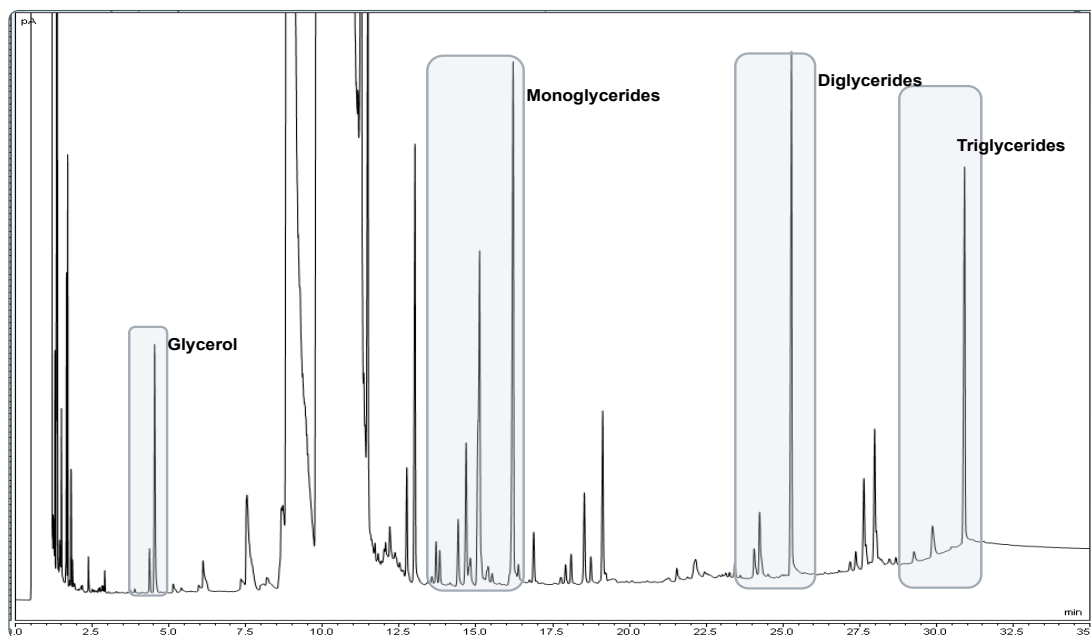
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## Process Monitoring

### Biodiesel Monitoring by GC-FID According to EN 1405



This is a chromatogram of a biodiesel sample analyzed with a [GC Flame Ionization Detector](#) (FID) according to the European Standards Organization's EN 14105. The areas where glycerol, monoglycerides, diglycerides, and triglycerides were detected are highlighted.

Download Application Note 10215: Determination of Free and Total Glycerin in Pure Biodiesel (B100) by GC in Compliance with EN 14105

Download Application Note 10212: Determination of Total FAME and Linolenic Acid Methyl Ester in Pure Biodiesel (B100) by GC in Compliance with EN 14103

Download Application Note 10192: Determination of Free and Total Glycerin in B-100 Biodiesel via Method ASTM D6584



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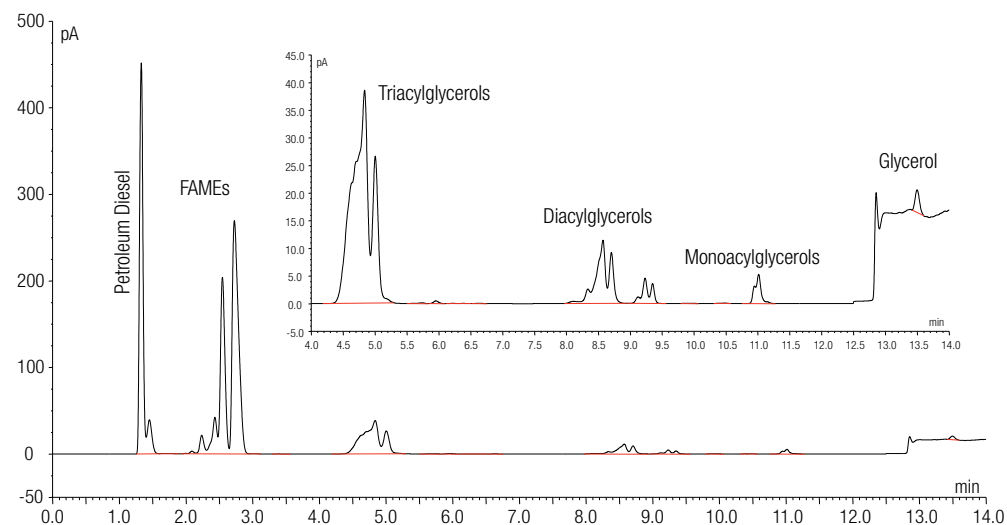
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### Acylated and Free Glycerols by HPLC-CAD with ASTM D6584



A simple, normal-phase method using the [UltiMate 3000 HPLC system](#) with the Thermo Scientific™ Dionex™ [Corona™ ultra RS™](#) CAD provides a fast, accurate measurement of all acylated and free glycerols in a single analysis. In-process biodiesel, finished B100, and mixed petroleum biodiesel (20% biodiesel and 80% petrodiesel, or B20) can be diluted and directly analyzed in under 25 minutes and quantified to the current American Standard Test Method (ASTM) D6584 specifications.

Download Application Note 1049: A Single Method for the Direct Determination of Total Glycerols in All Biodiesels Using LC and Charged Aerosol Detection

Download Poster Note 70533: An Improved Global Method for the Quantitation and Characterization of Lipids by HPLC and Corona CAD

Download Application Bulletin 118: Determination of Residual Acylglycerols in Biodiesel



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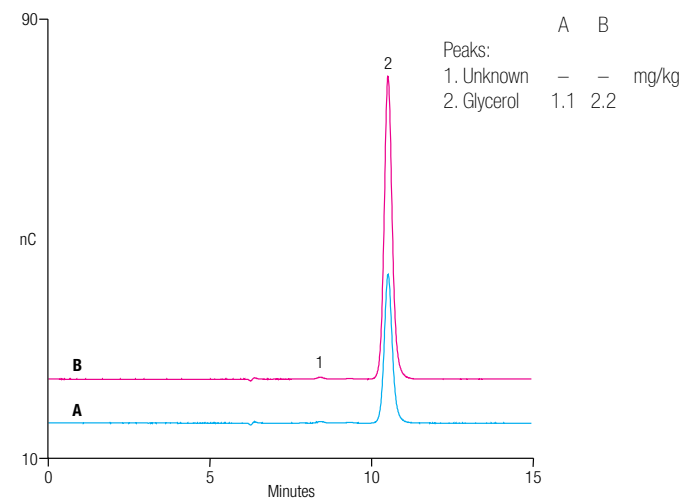
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## Process Monitoring Analysis of Free Glycerol in B100 using IC-PAD

HPAE-PAD is another well-established method that can determine carbohydrates and glycols without sample derivatization. The Dionex [CarboPac MA1](#) Column provides the selectivity that allows glycerol to be retained longer on this column than on other columns, resulting in the resolution of glycerol from other compounds, and the determination of free and total glycerol in a biodiesel sample. Shown here are results for free glycerol of a B100 soy sample A) without, and B) with added glycerol (labeled peak 1).



Download Application Note 255: Determination of Free and Total Glycerol in Biodiesel Samples by HPAE-PAD Chromatography

Download Application Note 188: Determination of Glycols and Alcohols in Fermentation Broths Using Ion-Exclusion Chromatography and Pulsed Amperometric Detection

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# Biodiesel



## Quality Control

Meeting regulatory requirements for methanol and quantifying elements such as sodium and potassium in biodiesel fuels is critical. Analysis needs to be fast, accurate, and reliable to ensure optimal output.

- HPLC-CAD
- IC
- NIR
- ICP-OES



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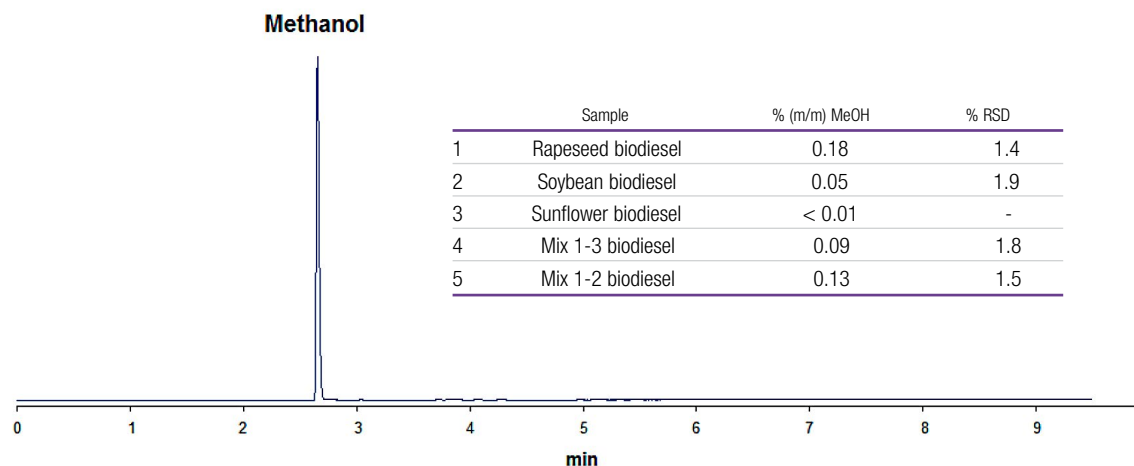
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## Quality Control Acylated and Free Glycerols by HPLC-CAD with ASTM D6584



Finally, repeatability of the assay was tested by performing five consecutive injections for each biodiesel sample, resulting in excellent performance.

Results from the analysis of methanol in pure biodiesel (B100) by headspace GC, according to EN 1410, show a pure sample. The table demonstrates the repeatability of the test by performing 5 consecutive injections for 5 biodiesel samples, showing excellent performance. Units are expressed as mass/mass or m/m.

[Download Application Note 1049: A Single Method for the Direct Determination of Total Glycerols in All Biodiesels Using LC and Charged Aerosol Detection](#)

[Download Poster Note 70533: An Improved Global Method for the Quantitation and Characterization of Lipids by HPLC and Corona CAD](#)

[Download Application Bulletin 118: Determination of Residual Acylglycerols in Biodiesel](#)



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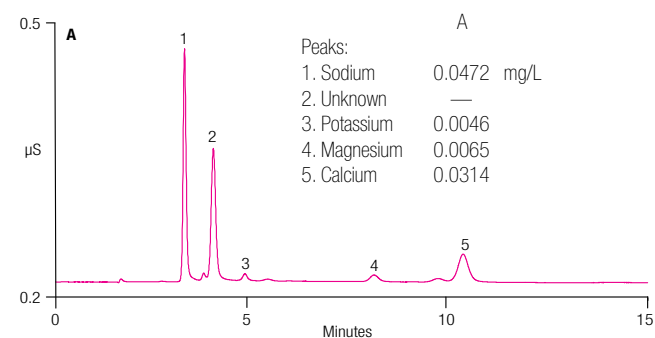
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## Quality Control Analysis of Group I and II Metals in Biodiesel by IC

In this chromatogram of a B99 (99% biodiesel and 1% petroleum diesel) and B20 extraction, the four cations are well resolved from one another and easily quantified in less than 15 minutes using a Dionex [IonPac CS12A Column](#). The combined sodium and potassium concentration determined in B99 was 0.991 mg/mL with a combined concentration of magnesium and calcium concentration of 0.207 mg/mL, both of which are well below the ASTM limits.



Download Application Note 203: Determination of Cations in Biodiesel Using a Reagent-Free Ion Chromatography System with Suppressed Conductivity Detection



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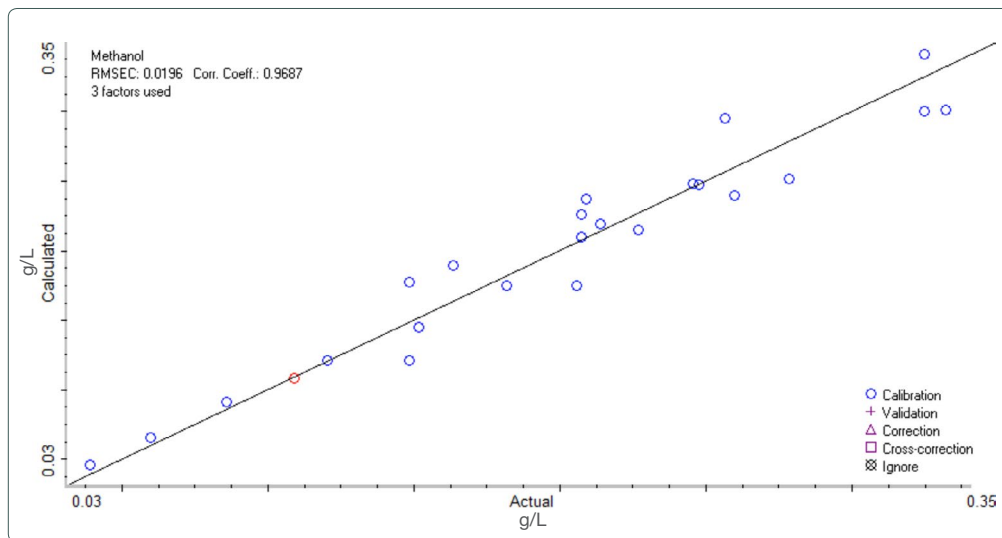
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## Quality Control

### Calibration Curve for Measurement of Methanol by NIR



This slide shows results of the NIR Partial Least Squares (PLS) calibration curve for methanol. Results demonstrate the capability of NIR to continuously monitor the biodiesel transesterification process in real-time.

[View the Webinar: Near Infrared Analysis of Biofuels](#)



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## Quality Control Elemental Analysis of Biodiesel by ICP-OES

	Europe	USA	Actuals (recovery)
Specification	EN 14214	ASTM D6751	
Sulfur (ppm)	10 max	15 max	5.54
Phosphorous (ppm)	10 max	10 max	0.72
Group I metals (Na & K)	5 max (combined)		0.15
Group II metals (Ca & Mg)	5 max		0.03

Results from the analysis of P, S, and Group I and II metals shows [ICP](#) can clearly measure these elements to limits below US and European regulatory specifications.

Download Application Note 40967: Petrochemical Series – Analysis of Biodiesel using the iCAP 6000 series ICP



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Analyte		Application
<b>Biodiesel</b>	AN 40967:	<i>Analysis of Biodiesel Using the iCAP 6000 Series ICP</i>
<b>Biomass</b>	AN 363:	<i>Using Accelerated Solvent Extraction in Alternative Fuel Research</i>
<b>Carbohydrates</b>	AN 282: LPN 2827-01:	<i>Rapid and Sensitive Determination of Biofuel Sugars by Ion Chromatography</i> <i>Methods for Determining Sugars and Hydroxymethylfurfural in Biomass</i>
<b>Carbohydrates, Lipids</b>	LPN 2168-01:	<i>Analysis of Carbohydrates and Lipids in Microalgal Biomass with HPAE-MS and LC/MS</i>
<b>Chloride, Sulfate</b>	AN 290: AN 297:	<i>Determination of Total and Potential Sulfate and Total Chloride in Ethanol According to ASTM Method D 7319</i> <i>Determination of Total and Potential Sulfate and Total Chloride in Fuel Grade Butanol Per ASTM D7319-09</i>
<b>FAME</b>	AN 51258:	<i>Biodiesel (FAME) Analysis by FT-IR</i>
<b>FAME, Linolenic Acid</b>	AN 10212:	<i>Determination of Total FAME and Linolenic Acid Methyl Ester in Pure Biodiesel (B100) by GC in Compliance with EN 14103</i>
<b>FAME, Monoacyl-, Diacyl-, and Triacylglycerols, Glycerol</b>	PN 70046:	<i>A Single Method for the Direct Determination of Total Glycerols in All Biodiesels Using Liquid Chromatography and Charged Aerosol Detection</i>
<b>Glycerin</b>	AN 10192: AN 10215:	<i>Determination of Free and Total Glycerin in B-100 Biodiesel via Method ASTM D6584</i> <i>Determination of Free and Total Glycerin in Pure Biodiesel (B100) by GC in Compliance with EN 14105</i>
<b>Glycerol</b>	AN 255: AN 1049: AN 51853:	<i>Determination of Free and Total Glycerol in Biodiesel Samples by HPAE-PAD Chromatography</i> <i>A Single Method for the Direct Determination of Total Glycerols in All Biodiesels Using Liquid Chromatography</i> <i>Determination of Free Glycerol in Biodiesel with the Evolution Array UV-Visible Spectrophotometer</i>
<b>Group I &amp; II Metals</b>	AN 203:	<i>Determination of Cations in Biodiesel Using a Reagent-Free Ion Chromatography System with Suppressed Conductivity Detection</i>
<b>Lipids, Glycerol, Methanol</b>	AN 51544:	<i>Trace Contaminant Analysis in Biodiesel with an Antaris II FT-NIR Analyzer</i>
<b>Methanol</b>	AN 10216:	<i>Determination of Methanol Content in Pure Biodiesel (B100) by Headspace-GC in Compliance with EN 14110</i>
<b>Oil Content</b>	CAN 301:	<i>Determination of Oil Content in Biodiesel Feedstock by Accelerated Solvent Extraction</i>
<b>Sulfur</b>	AN 42164: AN PI2044.0207:	<i>Determination of Sulfur in ULSD, Biodiesel and Jet Fuel using a Thermo Scientific PRO 5000 Series Analyzer According to ASTM D5453</i> <i>Trace Sulfur Analysis in the Production of Biofuels</i>
<b>Wood Hydrolysates</b>	AN 1089 AN 1091	<i>Determination of Carbohydrates in Acid Hydrolysates of Wood</i> <i>Determination of Uronic Acids and Wood Sugars in Wood-Based Hydrolysates</i>

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