

Improving HPLC Performance with Alltech's Evaporative Light Scattering Detector (ELSD)

1

Application Booklet #1 Pharmaceutical Analyses



- **Nutraceuticals**

- Ginkgo Biloba
- Ginsenosides
- Catechins



- **Pharmaceuticals**

- Antibiotics
- Combinatorial Chemistry
- Steroids



- **Excipients**

- Polymers and Surfactants
- Carbohydrates
- Lipids

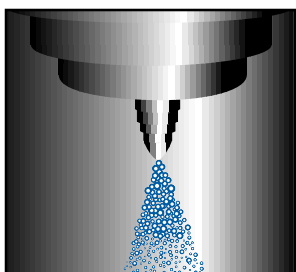
Introduction

Powerful Detection for Pharmaceutical Analysis

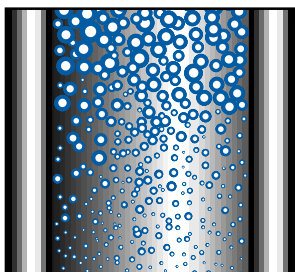
Alltech's ELSD is ideal for analyzing many different pharmaceutical compounds. Its response is based on the concentration of the analyte, whether or not it absorbs UV light. It determines sample composition even for unknowns where standards and structural data are not available. The ELSD's baseline is quiet and free from drift during gradients. The result? High sensitivity and accurate quantitation.

ELSD Detection Principles - Universal, Versatile, Sensitive

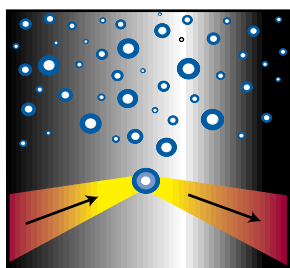
Evaporative Light Scattering Detectors use a simple three-step process that produces a signal for any non-volatile sample component:



1. Nebulization: Inside the nebulizer, the column effluent passes through a needle, mixes with nitrogen gas, and forms a dispersion of droplets.



2. Mobile Phase Evaporation: The droplets pass through a heated "drift tube" where the mobile phase evaporates leaving a fine mist of dried sample particles in solvent vapor.



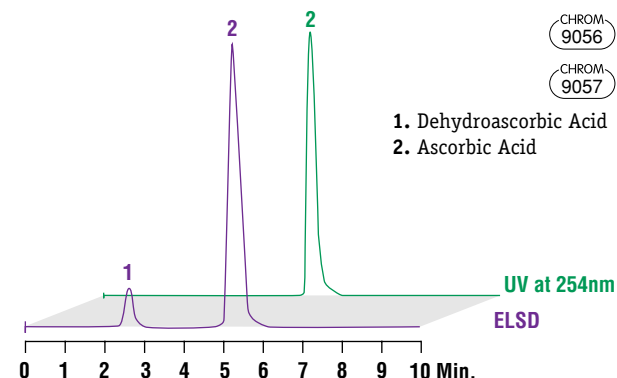
3. Detection: The sample particles pass through a flowcell where they are hit with a laser light beam. Light scattered by the sample particles is detected, generating an electrical signal.

This unique detection method is the key to the ELSD's versatility and performance. Since all particles scatter light, all samples are detected with high sensitivity and accuracy, regardless of their functional groups or optical properties. All samples are detected with nearly equivalent response factors, making concentration determination easier.

Near Equivalent Response for All Sample Types

The non-chromophoric degradant of ascorbic acid, dehydroascorbic acid, shows no response on the UV detector. Since the ELSD's response is independent of optical characteristics, both components are detected accurately.

Ascorbic Acid and Its Degradant, Dehydroascorbic Acid

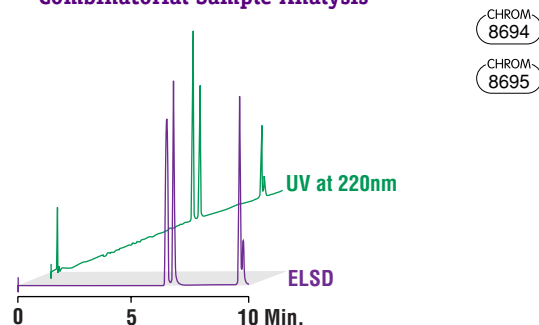


Column: Jordi C18-DVB, 5 μ m, 150 x 4.6mm
Mobile Phase: Water, pH 2.70 w/Acetic Acid:Acetonitrile: Methanol, 40:50:10
Flowrate: 0.7mL/min
Detector: Alltech ELSD

Maintain Stable Baselines During Gradients

Mobile phase modifiers cause baseline drift during gradient elution with low-wavelength UV detection. With the ELSD, the mobile phase is evaporated before detection, resulting in a stable baseline.

Combinatorial Sample Analysis



Column: Alltech Alltima™ C18, 5 μ m, 50 x 2.1mm
Mobile Phase: A: 0.1% Formic Acid in Water
B: 0.1% Formic Acid in Acetonitrile
Gradient: Time: 0 | 10 | 11
%B: 5 | 95 | 95
Flowrate: 0.5mL/min
Detector: Alltech ELSD



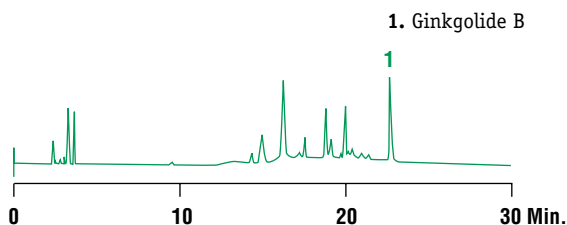
Alltech's ELSD. . .

. . .Provides Sensitive Detection

Ginkgoaceae leaf extracts treat cerebrovascular and peripheral circulatory disorders of the elderly. They are also used to treat asthma. Ginkgo contains ginkgolides, which are diterpenes that inhibit the platelet activation factor (PAF). Since ginkgolides have poor UV absorption and have a low concentration in leaves, ELSD is an ideal detection method.

Ginkgo Biloba Extract

CHROM-9019



Column: Alltech Adsorbosphere® C8, 5µm, 250 x 4.6mm
Mobile Phase: A: 0.1% TFA in Acetonitrile:Water (5:95)
 B: 0.1% TFA in Acetonitrile:Water (70:30)
Gradient:

Time:	0	30	35	40
%B:	0	75	75	0

Flowrate: 1.0mL/min
Detector: Alltech ELSD

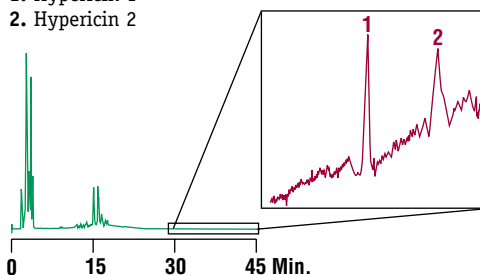
. . .Detects Low-levels

St. John's Wort treats neuralgia, anxiety, and nervous tension. It is an alternative to Selective Serotonin Reuptake Inhibitors. The ELSD detects Hypericin (Naphthodianthrone), the herb's active ingredient.

St. John's Wort

CHROM-9020

1. Hypericin 1
2. Hypericin 2



Column: Alltech Adsorbosphere® C8, 5µm, 250 x 4.6mm
Mobile Phase: A: 0.1% TFA in Acetonitrile:Water (5:95)
 B: 0.1% TFA in Acetonitrile:Water (70:30)
Gradient:

Time:	0	40	50	55
%B:	0	100	100	0

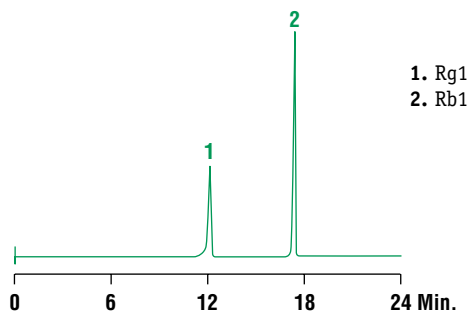
Flowrate: 1.0mL/min
Detector: Alltech ELSD

. . .Eliminates Low-Wavelength UV Detection Problems

The root of Panax Ginseng has been used in oriental medicine since ancient times as a stimulant, tonic, diuretic and digestive aid. Because ginsenosides are poor chromophores, they are only detected at low-wavelengths, which prevents the use of gradient elution. The ELSD's universal detection quantifies ginsenosides accurately, even with gradient elution.

Ginsenosides

CHROM-8868



Column: Whatman Partisil® ODS-3, 5µm, 250 x 4.6mm
Mobile Phase: A: Water B: Acetonitrile
Gradient:

Time:	0	30
%B:	20	30

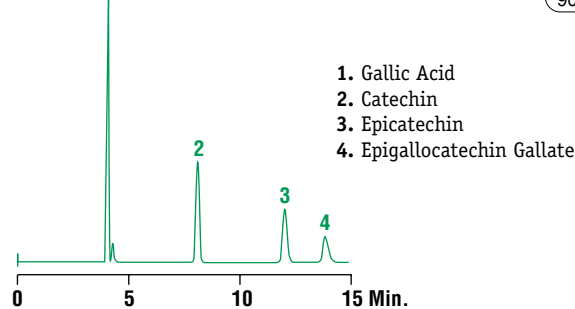
Flowrate: 0.8mL/min
Detector: Alltech ELSD

. . .Reduces Matrix Interferences

These flavonoids are astringents that treat hepatic diseases. Catechins are chromophoric, and therefore detectable by UV. However, the ELSD improves their analysis by minimizing matrix interferences. Since the ELSD's response is independent of the sample's optical characteristics, large concentrations of chromophoric sample components will not mask the analytes of interest.

Catechins in Green Tea

CHROM-9025



Column: Alltech Platinum® EPS C18, 5µm, 250 x 3.2mm
Mobile Phase: 0.05% TFA in Water:Acetonitrile (87:13)
Flowrate: 0.5mL/min
Detector: Alltech ELSD

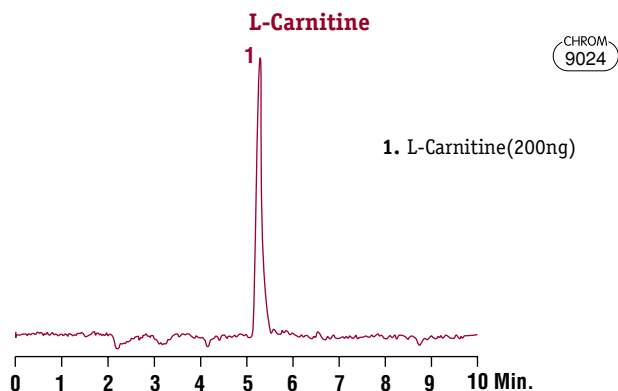


Pharmaceuticals

Alltech's ELSD. . .

. . .Works with Volatile Ion-Pair Reagents

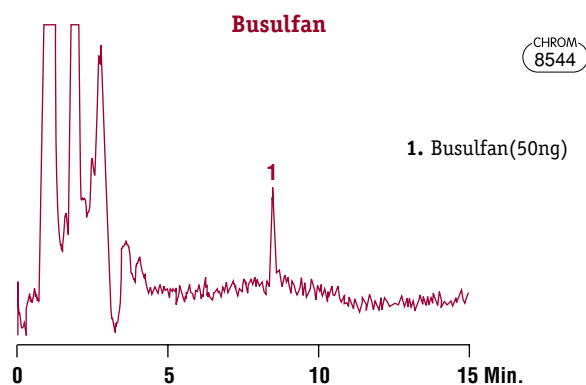
L-Carnitine is an amino acid found in nutritional supplements and pharmaceutical formulations. Its primary function is to regulate fat metabolism. Analysis of L-Carnitine is difficult because it does not retain well on a reversed-phase column and is non-chromophoric. Using a volatile ion pair reagent and the ELSD, L-Carnitine is separated and detected with exceptional sensitivity.



Column: Alltech Alltima™ C18, 5µm, 250 x 2.1mm
Mobile Phase: 0.4% Pentafluoropropionic Acid in 40mM Ammonium Formate: 0.4% Pentafluoropropionic Acid in Methanol (85:15)
Flowrate: 0.2mL/min
Detector: Alltech ELSD

. . .Detects Non-chromophoric Compounds

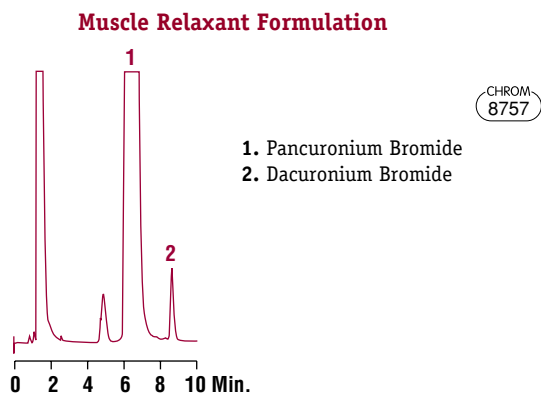
Busulfan is part of a group of drugs known as "alkylating" agents. They are used in the treatment of various cancers to slow or stop the growth of cancer cells. Like many new drugs, busulfan is non-chromophoric, making detection difficult. Using a normal-phase HPLC method and the ELSD, busulfan is easily detected at low concentrations.



Column: Alltech Allsphere™ Silica, 3µm, 100 x 4.6mm
Mobile Phase: Hexane:Ethanol (75:25)
Flowrate: 1.0mL/min
Detector: Alltech ELSD

. . .Detects All Compounds with Near Equal Response

The neuromuscular blocking substance Pancuronium Bromide, and its degradation product Dacuronium Bromide, have low UV absorptivity. Since the ELSD response does not depend on the analyte's optical characteristics, it detects both the parent drug and the degradant. Direct quantification from peak area ratios is possible.



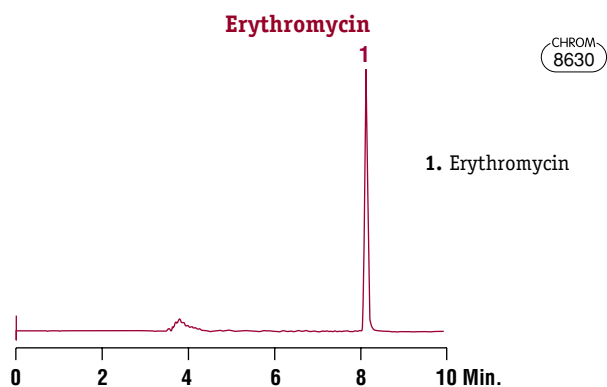
Column: Alltech Adsorbosphere® UHS C18, 150 x 3.2mm
Mobile Phase: A: 0.1% TFA in Water B: 0.1% TFA in Methanol
Gradient:

Time:	0	2	12
%B:	35	35	57

Flowrate: 0.5 mL/min
Detector: Alltech ELSD

. . .Eliminates Derivatization

The antibiotic erythromycin and other macrolides are easily detected with the ELSD. These compounds typically require derivatization for fluorescence or UV detection. Since the ELSD detects any sample less volatile than the mobile phase, derivatization is not required. Sample analysis time is reduced and sources of error are minimized.



Column: Alltech Macrosphere™ 300 C4, 7µm, 250 x 4.6 mm
Mobile Phase: Acetonitrile:0.2M Ammonium Acetate in Water, pH 4.5 (95:5)
Flowrate: 1.0 mL/min
Detector: Alltech ELSD



Alltech's ELSD. . .

. . .Accurately Represents Sample Composition

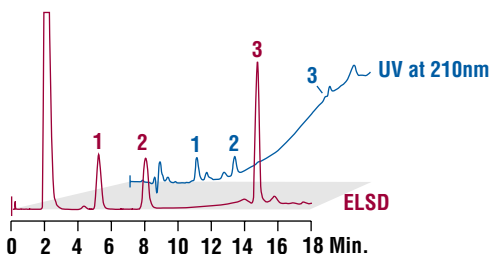
Unlike the estradiol conjugates, the pregnediol conjugate has no aromatic ring in its structure, causing unequal response for equal concentrations of the three analytes by UV detection. The ELSD gives a closer representation of sample composition because the ELSD's response does not depend on the sample's optical characteristics.

Steroid Conjugates

1. Estrone 3-Sulphate (100ng)
2. β -Estradiol 17-(β - α -Glucuronide) (100ng)
3. 5 β -Pregnane-3 α -2 α -Diol Glucuronide (220ng)

CHROM
7940

CHROM
7941



Column: Alltech Alltima™ C18, 5 μ m, 150 x 2.1mm
Mobile Phase: A: 0.1% TFA in Water B: 0.1% TFA in Methanol
Gradient:

Time:	0	10	18
%B:	60	100	100

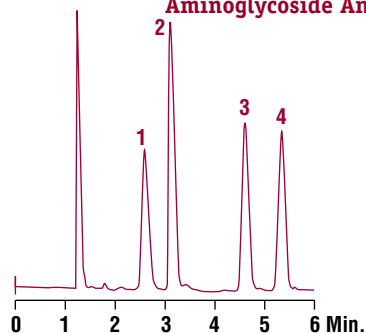
Flowrate: 0.2mL/min

. . .Eliminates Pre- or Post-Column Derivatization

Aminoglycoside antibiotics are traditionally analyzed using pre- or post-column derivatization followed by fluorescence detection. The ELSD eliminates the need for derivatization because its response is not dependent on the presence of fluorophores.

Aminoglycoside Antibiotics

CHROM
8948



1. Streptomycin
2. Amikacin
3. Tobramycin
4. Neomycin

Column: Alltech Alltima™ C18, 5 μ m, 150 x 4.6mm
Mobile Phase: A: 0.2% (v/v) Pentafluoropropionic Acid
 B: Methanol
Gradient:

Time:	0	10
%B:	45	65

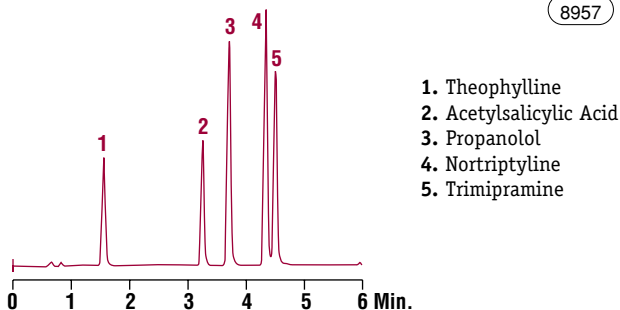
Flowrate: 1.0mL/min
Detector: Alltech ELSD

. . .Increases Accuracy in High Throughput Analyses

Assessing lead drug purity is more accurate using the ELSD compared to UV. The ELSD's signal closely reflects the sample's mass balance. The ELSD's universal detection capabilities and gradient compatibility are ideal for today's high throughput pharmaceutical applications.

High Throughput Analysis

CHROM
8957



1. Theophylline
2. Acetylsalicylic Acid
3. Propanolol
4. Nortriptyline
5. Trimipramine

Column: Alltech Alltima™ C18, 3 μ m, 53 x 7mm Rocket™
Mobile Phase: A: 0.1% TFA in Water B: 0.1% TFA in Acetonitrile
Gradient:

Time:	0	6
%B:	10	90

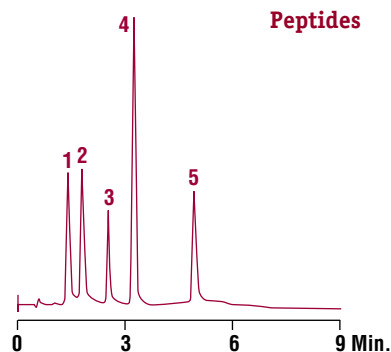
Flowrate: 2.5mL/min
Detector: Alltech ELSD

. . .Simplifies Peptide Analysis

Peptides are both powerful therapeutic agents and biomedical research tools. Baseline drift can be a problem when analyzed by low-wavelength UV. The ELSD's universal response and stable baseline make peptide analysis straightforward.

Peptides

CHROM
8874



1. (Arg-8)-Vasotocin
2. (Lys-8)-Vasopressin
3. Oxytocin
4. Leucine Enkephalin
5. Angiotensin 1

Column: Alltech Platinum™ EPS C18, 3 μ m, 53 x 7mm Rocket™
Mobile Phase: A: 0.15% TFA in Water
 B: 0.13% TFA in Acetonitrile:Water (95:5)
Gradient:

Time:	0	10
%B:	20	50

Flowrate: 3.0mL/min
Detector: Alltech ELSD

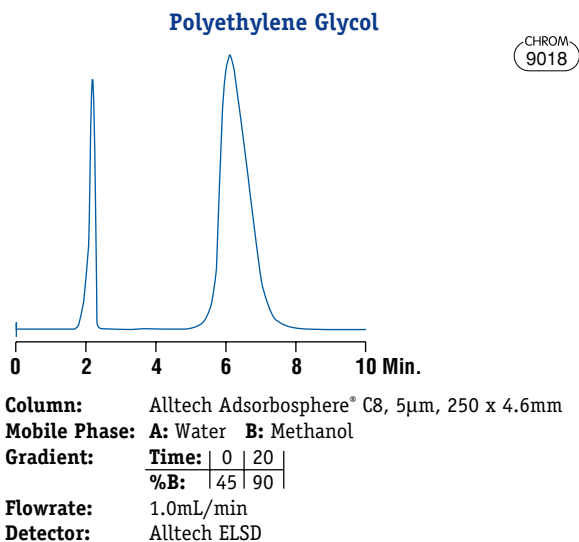


Excipients

Alltech's ELSD. . .

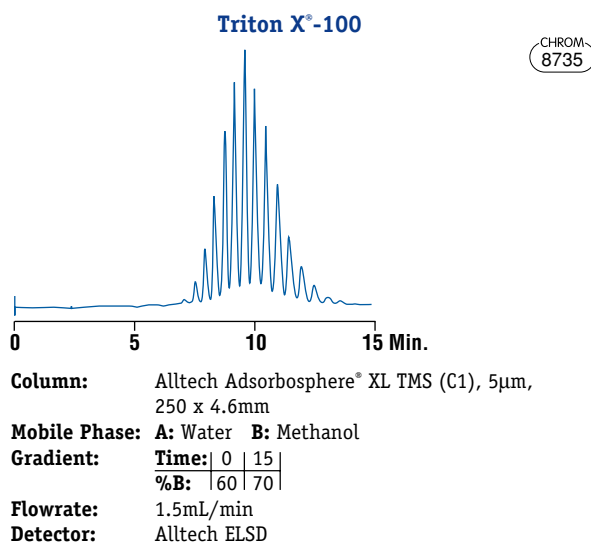
. . .Simplifies Detection of PEGs

Polyethylene Glycols (PEGs) are used in pharmaceutical formulations because of their unique solvent properties, low toxicity, blandness, and water solubility. UV detectors show no response to PEGs because the polymer does not absorb UV light. RI detectors have low sensitivity and long run times because they are not gradient compatible. The ELSD efficiently detects PEGs.



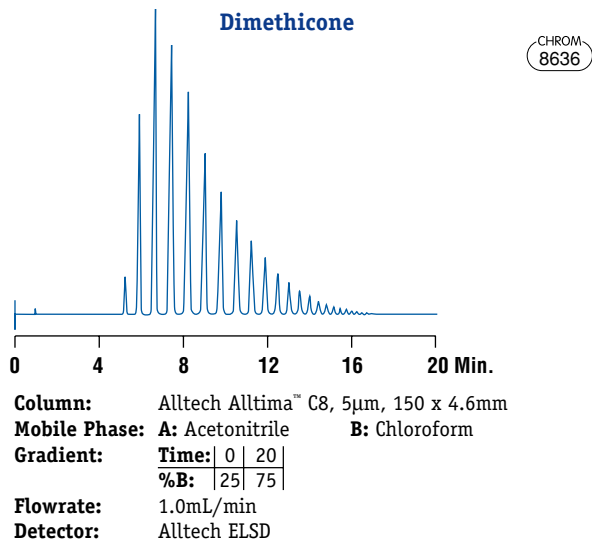
. . .Detects Both Chromophoric and Non-Chromophoric Compounds

Triton X®-100 is a non-ionic surfactant used as an emulsifier, dispersant, and stabilizer. Typical surfactant formulations may contain a variety of commercial products that are chromophoric and non-chromophoric. The ELSD detects all of these compounds regardless of their ability to absorb UV light.



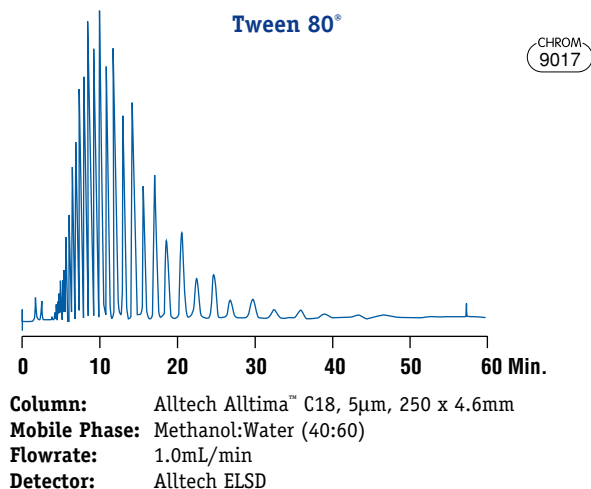
. . .Easily Detects Difficult Samples

Dimethicone is an inert, non-toxic, silicone-derived oil used as an antifoaming agent and as a spreading agent for creams and ointments. Dimethicone is difficult to analyze by HPLC because it is non-chromophoric and depending on its viscosity, has limited solubility. As a universal detector, the ELSD detects dimethicone easily.



. . .Provides Stable Baselines and Eliminates Solvent Fronts

Tween 80® is a hydrophilic non-ionic surfactant used to prepare oil-in-water emulsions. This non-chromophoric compound is difficult to detect by UV. Unstable baselines and solvent fronts make RI detection unsuitable. The ELSD easily separates and detects this non-chromophoric surfactant.

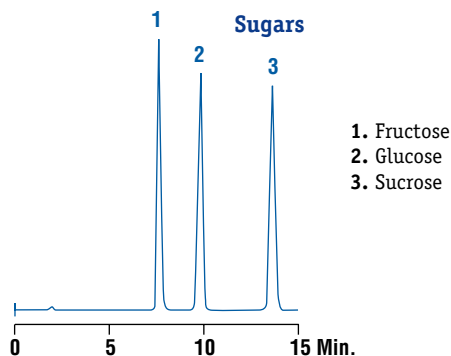




Alltech's ELSD. . .

. . .Provides Stable Baselines, High Sensitivity, and Gradient Compatibility

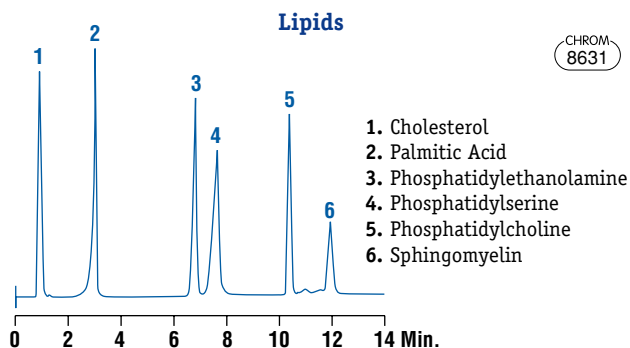
Carbohydrates are used in pharmaceutical formulations as bulking agents, binders, and flavoring agents. Their non-chromophoric nature precludes UV-Vis detection. RI detection is plagued by unstable baselines and low sensitivity. The ELSD is the best detection method for carbohydrates, delivering high sensitivity, stable baselines, and gradient compatibility.



Column: Astec Polymer NH₂, 5μm, 250 x 4.6mm
Mobile Phase: Acetonitrile:Water (75:25)
Flowrate: 1.0mL/min
Detector: Alltech ELSD

. . .Detects Non-chromophoric Compounds with Excellent Sensitivity

Lipids are present in pharmaceutical formulations as solubilizers, stabilizers, moisturizers, lubricants, viscosity modifiers, and emollients. They are difficult to detect by UV because they lack good chromophores. The ELSD responds to all lipids regardless of optical characteristics, while providing excellent sensitivity.



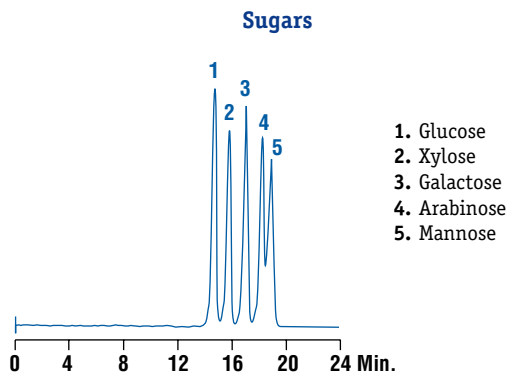
Column: Alltech Allsphere™ Silica, 3μm, 100 x 4.6mm
Mobile Phase: A: Isopropanol B: Hexane C: Water
Gradient:

Time:	0	7	15
%B:	40	40	40
%C:	2	8	8

Flowrate: 1.25mL/min
Detector: Alltech ELSD

. . .Eliminates Derivatization

Carbohydrates are important in many biological processes including molecular signaling and recognition, cell adhesion, viral and bacterial infections, and disease processes. Carbohydrate-based drugs and combinatorial libraries are difficult to detect using UV or RI detectors. With the ELSD, carbohydrates are easily detected without derivatization.

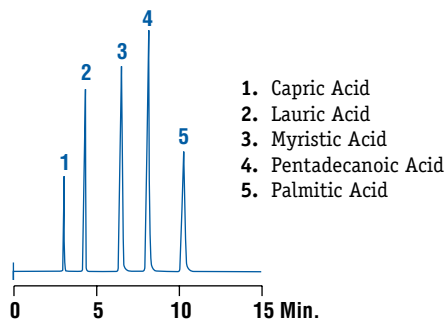


Column: Benson Carbohydrate BC-100, PB⁺, 10μm, 300 x 7.8mm
Mobile Phase: 100% Water
Flowrate: 0.5mL/min
Detector: Alltech ELSD

. . .Improves Sensitivity and Resolution

Since fatty acids lack chromophores, they are typically derivatized before analysis. RI detection cannot be used because complex mixtures of fatty acids require gradient elution for optimal separation. The ELSD eliminates derivatization by responding to all compounds, regardless of their optical activity. Gradient compatibility optimizes resolution.

Underivatized Low Chain Fatty Acids



Column: Alltech Alltima™ C18, LL, 5μm, 250 x 2.1mm
Mobile Phase: A: Water B: Acetonitrile
Flowrate: 0.4mL/min
Gradient:

Time:	0	5	10	20
%B:	77	80	80	95

Detector: Alltech ELSD

See How the ELSD Can Help You with Your Application

For additional information on specific applications, request the following application notes by calling or writing the number on the inserted reply card.

Pharmaceuticals

E0006	Water Soluble Vitamins
E0007	Fat Soluble Vitamins
E0011	Amino Acid, L-Histidine Standard
E0027	Steroids
E0028L	Caffeine and Aspirin
E0031	Chiral Separations, Propanolol Hydrochloride
E0032	Chiral Separations, Warfarin Enantiomers
E0033-E0035	Anabolic Steroids
E0040	Underivatized Amino Acids
E0041	Peptides, G-Y, G-L-Y, K-D
E0042	Steroid Conjugates
E0046	Muscle Relaxants
E0047	SFC Steroid Analysis
E0049L	Pharmaceutical, Stimulants
E0050	Combinatorial Sample Analysis
E0051	Ginsenosides

Polymers & Surfactants

E0009	Polystyrene, 800 to 90K MW Standards
E0010	Non-Ionic Detergent, Glucopyranosides
E0013	Surfactant, Pluronic L-62
E0014	Surfactant, Plurafac B-26
E0015	Surfactant, DC-190 Co-Polymer
E0016	Surfactant, DC-193 Co-Polymer
E0017	Surfactant, DC-194 Co-Polymer
E0018	Surfactant, DC-5103 Co-Polymer
E0026L	Non-Ionic Surfactant - Triton X®-100
E0029	Polymer - Polyethylene Glycol
E0030	Non-Ionic Surfactant, Tergitol NP®-35
E0039	Surfactant, Nonylphenol Ethoxylate
E0048	Dimethicone

Carbohydrates

E0004	Carbohydrate Standards
E0012	Carbohydrates in Fruit Juices
E0019	Corn Syrup Oligomers
E0020	Carbohydrates in Honey and Molasses
E0021	Polysaccharides, Dextran T-10 & T-40
E0043	Galactose
E0044	Carbohydrates in Soy Infant Formula
E0052	Orange Juice and Apple Juice Carbohydrate Profiles

Lipids

E0001	Cholesterol Standard
E0002	Seed Oils, Cottonseed and Linseed
E0003	Triglycerides in Olive Oil
E0005	Triglycerides in Chapstick®
E0008, E0024	Phospholipids in Egg Yolk
E0025	Fatty Acids (Underivatized)
E0036	Phospholipids Class Standards
E0037	Monoglyceride Purity, Glycerol Monolaurate
E0038	Fatty Acid Methyl Esters
E0045	Fatty Acid Analysis

Miscellaneous

E0022	Reproducibility Data, n-Dodecyl, β-D Glucopyranoside
E0023	Quantitation of Unknown

Another Detection Problem Solved!

"Alltech's ELSD has allowed our lab to quantify very low levels of impurities that could not be observed with RI detection. It has also eliminated the problem of estimating absorbance coefficients of unknown compounds with UV detection. Alltech's technical service has been exceptional. We have received instrument and applications help in a very timely manner." - *Al Del Guercio, Ciba Vision*

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Alltech

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