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**

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.

**Ascorbic Acid and Its Degradant, Dehydroascorbic Acid**

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.

**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**

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**Combinatorial Sample Analysis**

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**Alltech’s ELSD. . .**

**. . .Provides Sensitive Detection**
Ginkgofolium 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**

Column: Alltech Adsorbosphere®, 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: 04 0 5 05 5
%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**

Column: Alltech Adsorbosphere®, 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: 04 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**

Column: Whatman Partisil® ODS-3, 5µm, 250 x 4.6mm
Mobile Phase: A: Water B: Acetonitrile
Gradient: Time: 0 | 30 | 20 | 30
%B: | 0 | 100 | 100 | 0
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**

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

Phone: 1-800-255-8324  Web: www.alltechweb.com
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

Muscle Relaxant Formulation

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

1. Pancuronium Bromide
2. Dacuronium Bromide

Erythromycin

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

1. Erythromycin

0 2 4 6 8 10 Min.
**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. 5β-Pregnane-3α-2α-Diol Glucuronide (220ng)

**Aminoglycoside Antibiotics**

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

**. . .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.

**High Throughput Analysis**

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.

**Peptides**

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.

**. . .Increases Accuracy in High Throughput Analyses**

**. . .Simplifies Peptide Analysis**
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.

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**Polyethylene Glycol**

Column: Alltech Adsorbosphere® C8, 5µm, 250 x 4.6mm  
Mobile Phase: A: Water  B: Methanol  
Gradient: Time: 0 | 20  |
% B: 45 | 90   
Flowrate: 1.0mL/min  
Detector: Alltech ELSD

**Triton X*-100**

Column: Alltech Adsorbosphere® XL TMS (C1), 5µm, 250 x 4.6mm  
Mobile Phase: A: Water  B: Methanol  
Gradient: Time: 0 | 15  |
% B: 60 | 70   
Flowrate: 1.5mL/min  
Detector: Alltech ELSD

**Dimethicone**

Column: Alltech Alltima™ C8, 5µm, 150 x 4.6mm  
Mobile Phase: A: Acetonitrile  B: Chloroform  
Gradient: Time: 0 | 20  |
% B: 25 | 75   
Flowrate: 1.0mL/min  
Detector: Alltech ELSD

**Tween 80°**

Column: Alltech Alltima™ C18, 5µm, 250 x 4.6mm  
Mobile Phase: Methanol:Water (40:60)  
Flowrate: 1.0mL/min  
Detector: Alltech ELSD
Excipients

**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.

**Sugars**

1. Fructose
2. Glucose
3. Sucrose

**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.

**Lipids**

1. Cholesterol
2. Palmitic Acid
3. Phosphatidylethanolamine
4. Phosphatidyserine
5. Phosphatidylcholine
6. Sphingomyelin

**Column:** Alltech Allsphere™ Silica, 3µm, 100 x 4.6mm
**Mobile Phase:** A: Isopropanol  B: Hexane  C: Water
**Gradient:** Time: 0 5 10 15 %B: 40 40 40 80 %C: 2 8 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.

**Sugars**

1. Glucose
2. Xylose
3. Galactose
4. Arabinose
5. Mannose

**Column:** Benson Carbohydrate BC-100, PB+2, 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 compatability optimizes resolution.

**Underivatized Low Chain Fatty Acids**

1. Capric Acid
2. Lauric Acid
3. Myristic Acid
4. Pentadecanoic Acid
5. Palmitic Acid

**Column:** Alltech Alltima™ C18, LL, 5µm, 250 x 2.1mm
**Mobile Phase:** A: Water  B: Acetonitrile
**Gradient:** Time: 0 5 10 15 %B: 77 80 80 95
**Flowrate:** 0.4mL/min
**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
- E0004 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 Relaxants
- E0047 SFC Steroid Analysis
- E0049L Pharmaceutical, Stimulants
- E0050 Combinatorial Sample Analysis
- E0051 Ginsenosides

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, Glyceryl Monolaurate
- E0038 Fatty Acid Methyl Esters
- E0045 Fatty Acid Analysis

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

Polymers & Surfactants
- E0009 Polystyrene, 800 to 90X 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

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 Associates, Inc., Corporate Headquarters
2051 Waukegan Road • Deerfield, IL 60015 USA • Phone: 847-948-8600 • Fax: 847-948-1078
Email: alltech@alltechemail.com • Web Site: http://www.alltechweb.com

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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
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- 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