

Analysis of pharmaceuticals using HPLC with evaporative light scattering detection

Application Compendium

Author

Graham Cleaver
Agilent Technologies, Inc.



Universal detection for pharmaceutical analysis

The Agilent 385-ELSD is an advanced evaporative light scattering detector that delivers subambient evaporation down to 10 °C, providing maximum sensitivity for compounds with significant volatility below ambient temperature. The 385-ELSD benefits from fast data output rates and extremely low dispersion for fast LC, and delivers a universal response down to the low-nanogram range for truly representative analysis. Reproducibility is better than 2% for improved consistency of results. The 385-ELSD offers real time gas management that eliminates solvent effects to give a constant response across a gradient. Control and digital data collection come as standard for multivendor platforms so there is no need for an analog to digital converter. On-the-fly adjustment of light source intensity can save time during a run. Being complementary to LC/MS, and offering unrivalled flexibility and sensitivity, the 385-ELSD is the evaporative light scattering detector of choice for pharmaceutical applications.

Evaporative light scattering detection involves a three stage process.

1. **Nebulization** – Using an inert gas stream to form a plume of uniformly sized droplets
2. **Evaporation of the eluent** – Generating a plume of nonvolatile solute particles
3. **Optical detection** – Where the intensity of scattered light is proportional to the mass of solute passing through the optical chamber

Nebulization

Efficient nebulization using low gas flow rates is a feature of the 385-ELSD. Independent nebulizer temperature control and digital gas flow control provide excellent stability and reproducibility. Baseline noise is minimized by the removal of any poorly nebulized eluent through a drain port.

Evaporation

The nebulized stream passes through an independently temperature-controlled evaporator tube where solvent is removed at low temperature, leaving the less volatile solute particles behind. The 385-ELSD features patented, gas-flow control technology with a short evaporator tube that gives an extremely low swept volume for minimal peak dispersion. This provides maximum resolution for high speed separations, which is especially important for work with small columns.

Optical detection

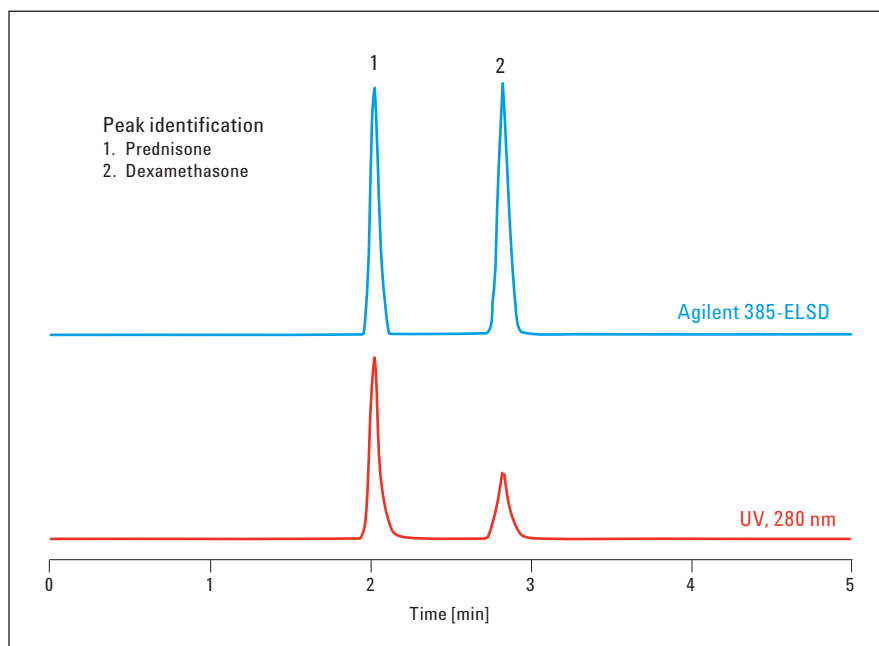
The solute particles are detected as they pass through the optical chamber. The high power LED and advanced design of the electronics deliver maximum sensitivity.

Benefits of ELS Detection

Obtain a more uniform response

The 385-ELSD is not dependent on a compound's optical properties so it provides a more uniform response than UV-Visible detection, making it the ideal detector for purity analysis or where calibration standards are not available.

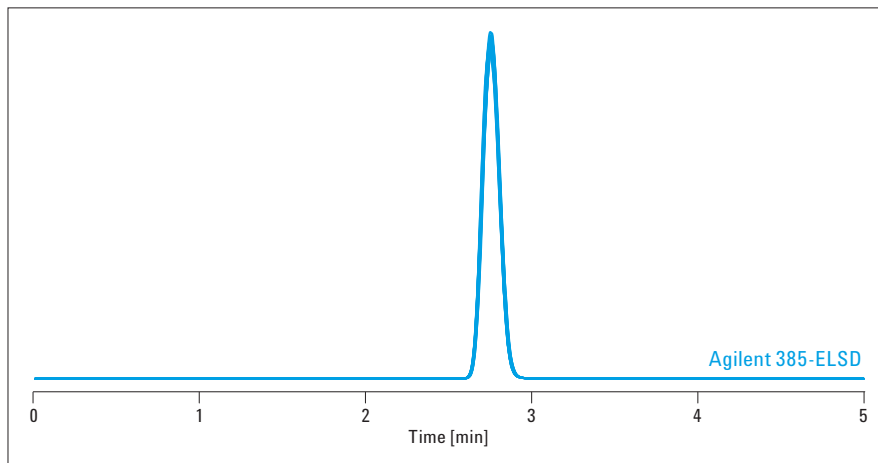
Column: Agilent Polaris C18,
150 × 4.6 mm, 5 µm
Eluent: Water:Acetonitrile 50:50
Flow rate: 1.0 mL/min
Injection volume: 10 µL
ELS detection: Agilent 385-ELSD
(neb. = 30 °C, evap. = 30 °C,
gas flow = 1.4 SLM)
UV detection: 280 nm



Achieve superb RSD – 50 caffeine injections

Excellent reproducibility below 2% gives reliable and accurate results – you can have complete confidence in your data.

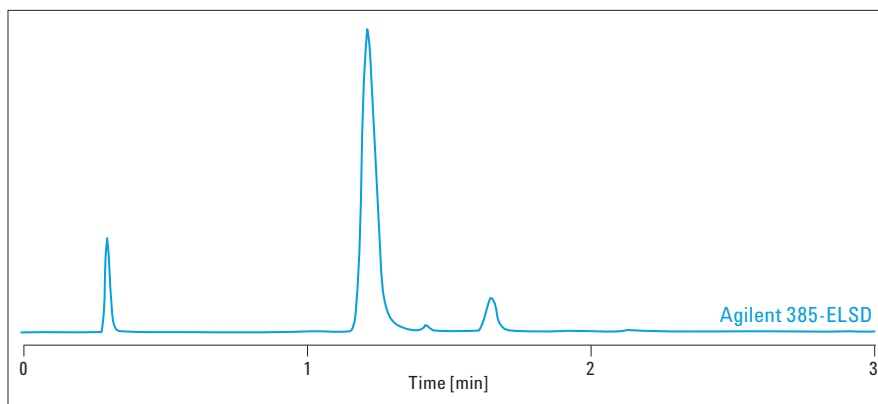
Column: Agilent Pursuit C18,
150 × 4.6 mm, 5 μm
Eluent: Water:Acetonitrile 40:60
Flow rate: 1.0 mL/min
Injection volume: 10 μL
ELS detection: Agilent 385-ELSD (neb. = 40 °C,
evap. = 40 °C,
gas flow = 1.4 SLM)



Achieve fast gradient and steep baselines

The excellent baseline stability across steep gradients and low dispersion characteristics combined with fast data output rates makes the Agilent 385-ELSD ideal for fast LC.

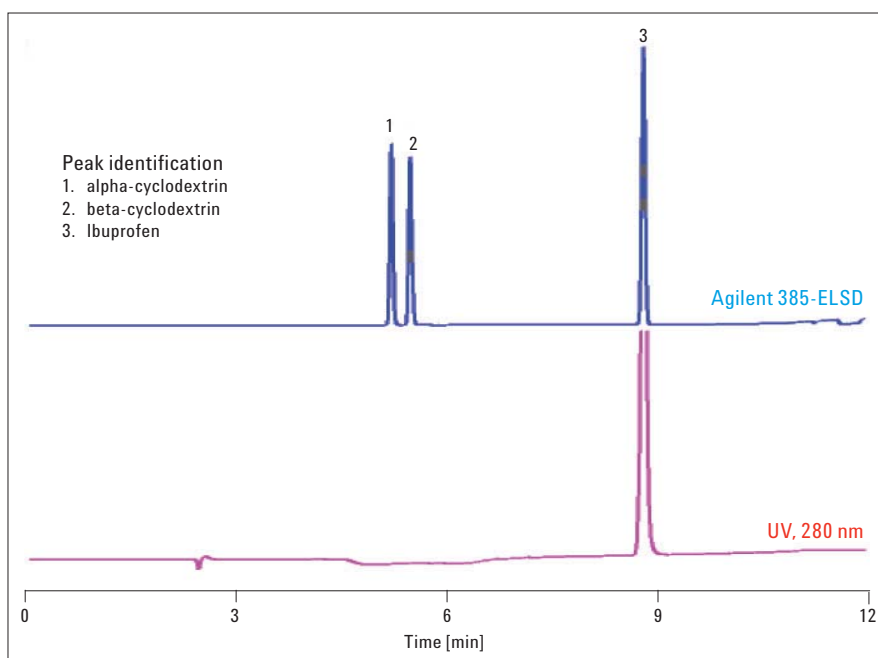
Sample: Tertiary aminols
Column: C18, 50 × 4.6 mm, 5 μm
Eluent A: Water + 0.1 % Formic acid
Eluent B: Acetonitrile + 0.1 % Formic acid
Gradient: 5–95 % B in 5 min
Flow rate: 2.5 mL/min
Injection volume: 10 μL
ELS detection: Agilent 385-ELSD (neb. = 40 °C,
evap. = 50 °C,
gas flow = 1.5 SLM)



Detect compounds with no UV chromophore

ELS detection is necessary for compounds that do not possess a UV chromophore, but require gradient elution, such as cyclodextrins. Cyclodextrins are commonly used with hydrophobic drug molecules to improve the target compound's solubility, stability, bioavailability, and dissolution. Consequently, their characterization is of great importance within the pharmaceutical sector.

Column: C18, 50 × 4.6 mm, 5 μm
Eluent A: Water
Eluent B: Acetonitrile
Gradient: 50–95 % B in 5 min
Flow rate: 1.0 mL/min
Injection volume: 20 μL
ELS detection: Agilent 385-ELSD (neb. = 30 °C,
evap. = 50 °C,
gas flow = 1.0 SLM)

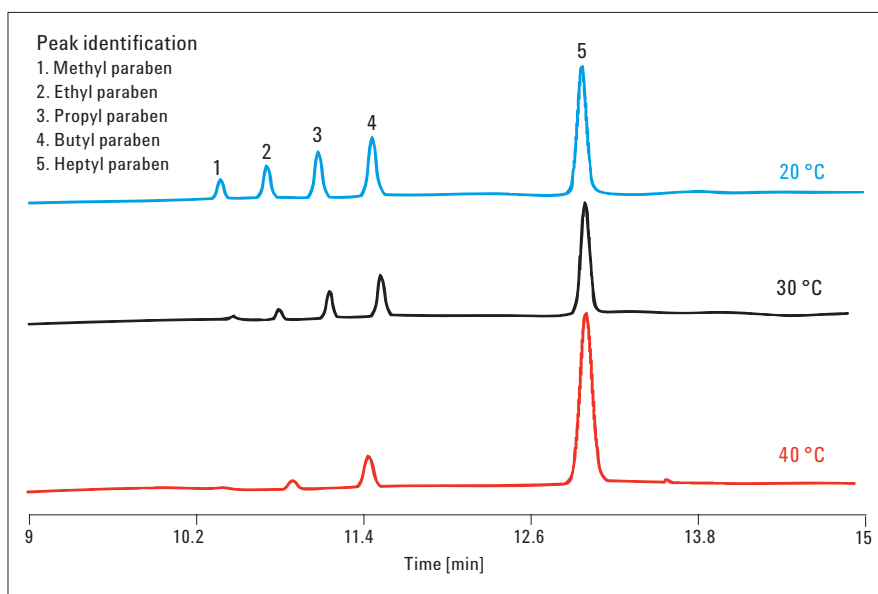


Sub-ambient ELS detection – Ideal for low molecular weight compounds

Achieve sub-ambient detection for semi-volatiles

The unique cooled evaporation zone of the Agilent 385-ELSD provides sub-ambient operation to deliver unrivalled detection of low molecular weight compounds not seen by other ELS detectors. Operating the 385-ELSD at 20 °C provides better detection of parabens, which are commonly used in pharmaceutical and cosmetic formulations.

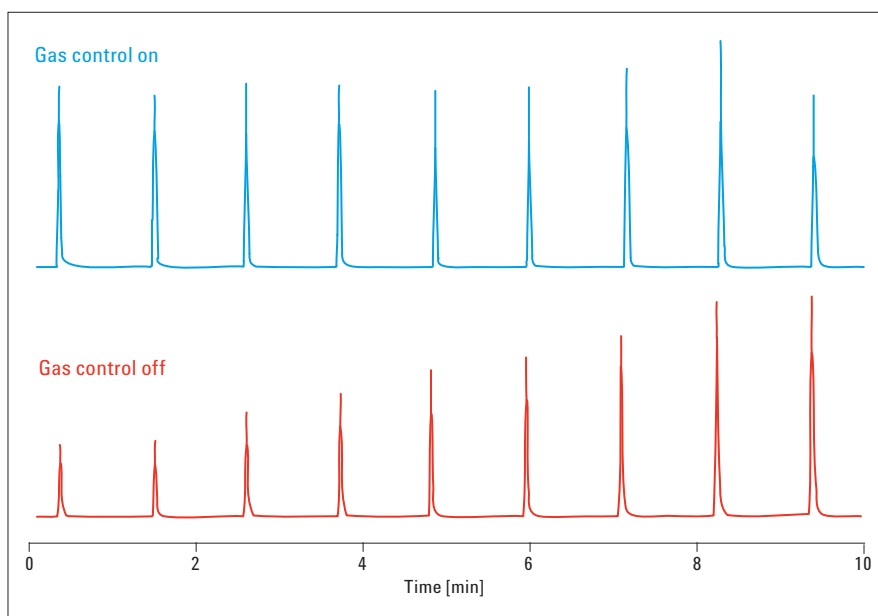
Sample: Parabens
Column: Agilent Pursuit C18,
150 × 2.1 mm, 5 µm
Eluent A: Water
Eluent B: Acetonitrile
Gradient: 5–70% B in 5 min,
70–95% B in 2 min
Flow rate: 0.2 mL/min
Injection volume: 10 µL
ELS detection: Agilent 385-ELSD
(neb. = 30 °C, as shown,
gas flow = 1.4 SLM)



Obtain an unrivalled uniform response across a gradient

The 385-ELSD is unique in its ability to control gas flows during an injection to produce a uniform detector response across a solvent gradient. This real-time benefit offers an alternative approach to mobile phase compensation when quantifying unknowns under gradient conditions.

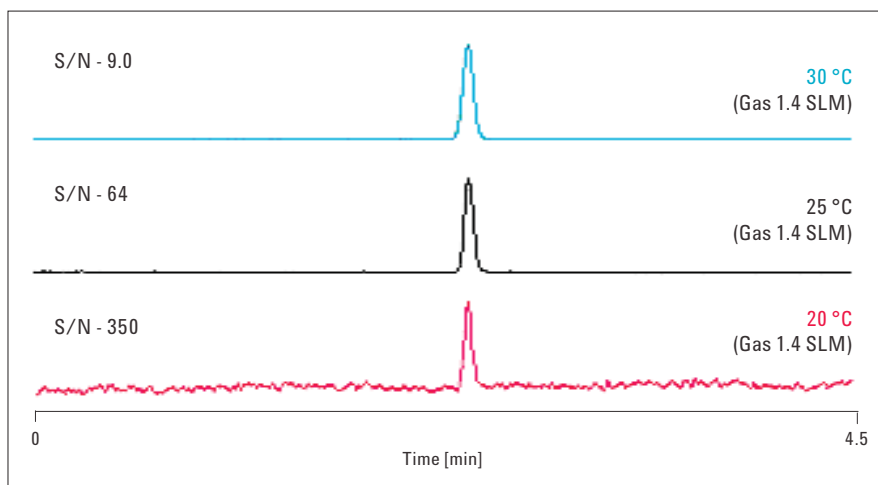
Column: Agilent Pursuit C18,
150 × 2.1 mm, 5 µm
Eluent A: Water
Eluent B: Acetonitrile
Gradient: 5–95% B in 10 min
Flow rate: 1.0 mL/min
Injection volume: 10 µL (every min)
Detection: Agilent 385-ELSD
(neb. = 30 °C, evap. = 30 °C)



Improve signal-to-noise using sub-ambient analysis

Polyethylene glycols of low molecular weight are used to improve the aqueous solubility of hydrophobic drugs. PEG 106 is semivolatile with no UV chromophore, so ELS detection is required. The Agilent 385-ELSD, operating at subambient temperatures, can significantly improve the detection of this compound as shown.

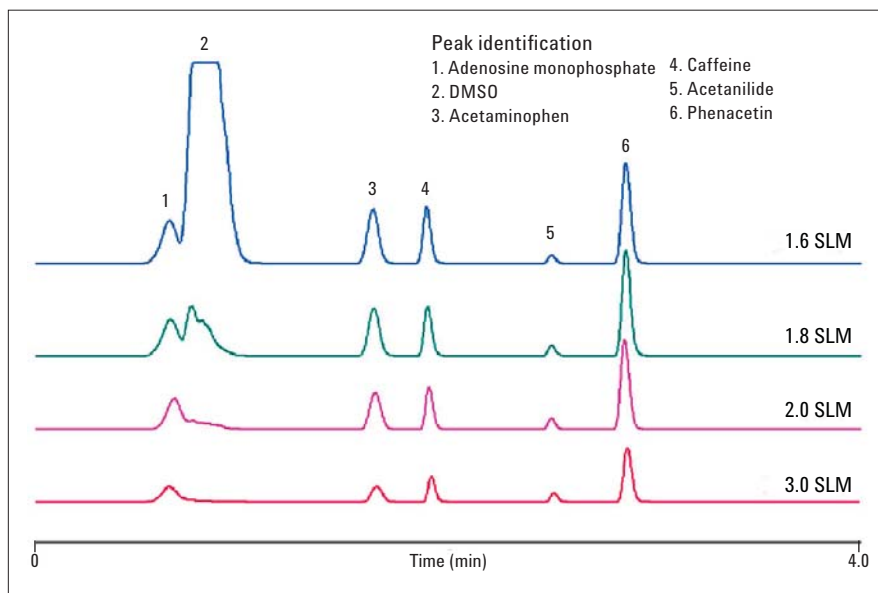
Column: C8, 50 × 4.6 mm, 5 μm
 Eluent A: Water
 Eluent B: Acetonitrile
 Gradient: 5–100 % B in 5 min
 Flow rate: 0.2 mL/min
 Injection volume: 1.0 μL
 Detection: Agilent 385-ELSD
 (neb. = 30 °C, evap. as shown)



Obtain DMSO transparency at 30 °C

The unique design of the 385-ELSD gives DMSO transparency at low temperatures by increasing gas flow, enabling the detection of fast eluting semi-volatile compounds. This allows faster gradients and increased sample throughput in combinatorial analysis.

Column: C8, 50 × 4.6 mm, 5 μm
 Eluent A: Water
 Eluent B: Acetonitrile
 Gradient: 5–100 % B in 5 min
 Flow rate: 0.2 mL/min
 Injection volume: 20 μL
 Detection: Agilent 385-ELSD
 (neb. = 25 °C, evap. = 30 °C, gas flow as shown)

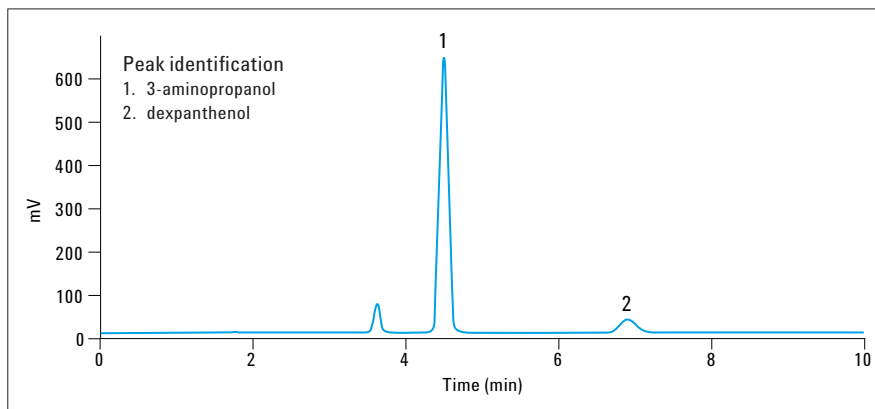


Analysis of pharmaceuticals with ELS detection

Deliver accurate compositional data

The Agilent 385-ELSD's uniform response delivers accurate composition information for pharmaceutical mixtures, such as dexpanthenol and 3-aminopropanol in eye drops.

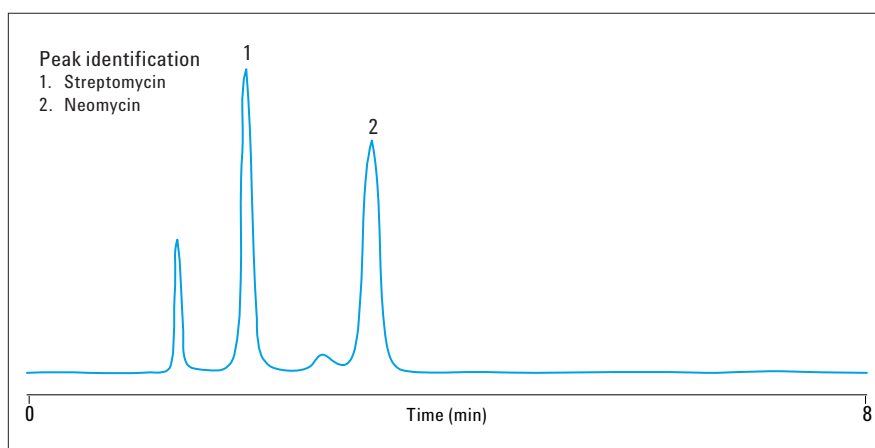
Column: C18, 150 × 4.6 mm, 5 µm
Eluent A: 0.1% HFBA in Water
Eluent B: 0.1% HFBA in CAN
Gradient: 95:5 A:B
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Detection: Agilent 385-ELSD (neb. = 30 °C, evap. = 60 °C, gas flow = 1.4 SLM)



Detect antibiotics with no UV chromophore

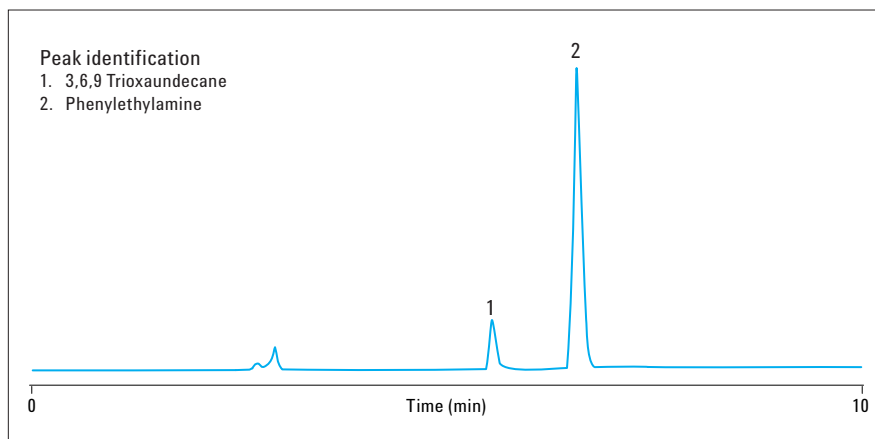
ELS detection is the ideal choice for the detection of aminoglycoside antibiotics because they are difficult to detect by UV due to their extremely weak chromophore. Antibiotics such as streptomycin and neomycin are extremely important in clinical medicine because of their efficacy and cost. However, owing to their potential toxicity, the accurate quantification of these antibiotics in pharmaceutical and medicinal preparations is vital.

Column: C18, 150 × 4.6 mm, 5 µm
Eluent A: 0.3 % Pentafluoropropionic acid (PFPA) in MeOH
Eluent B: 0.3% PFPA in 47 mM ammonium formate
Gradient: 45:55 A:B
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Detection: Agilent 385-ELSD (neb. = 40 °C, evap. = 85 °C, gas flow = 1.2 SLM)



Eliminate the need for derivatization

Phenylethylamine (PEA) is a low molecular weight amine that increases dopamine levels in the brain. While substituted phenylethylamines are used in a wide range of drugs from stimulants, hallucinogens, antidepressants and bronchodilators, ELS detection provides a fast and rapid means of analyzing PEAs in pharmaceutical mixtures without the need for derivatization.



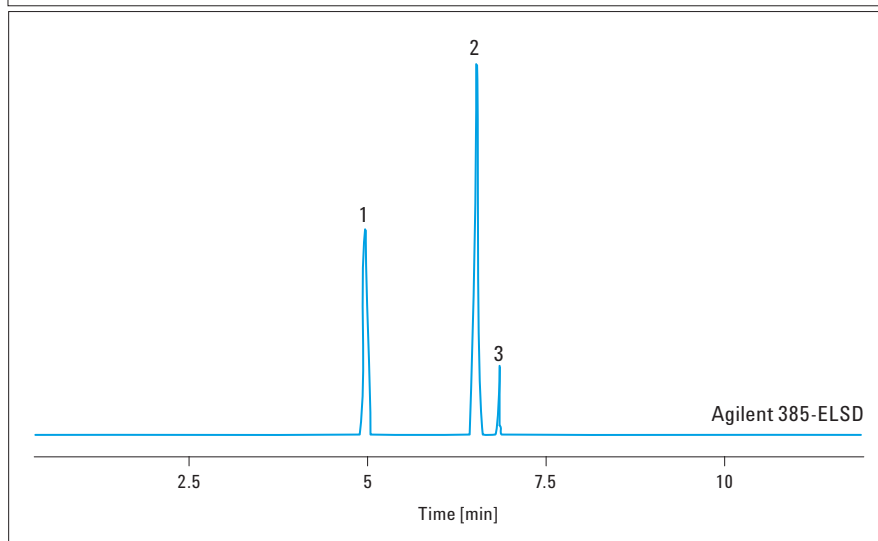
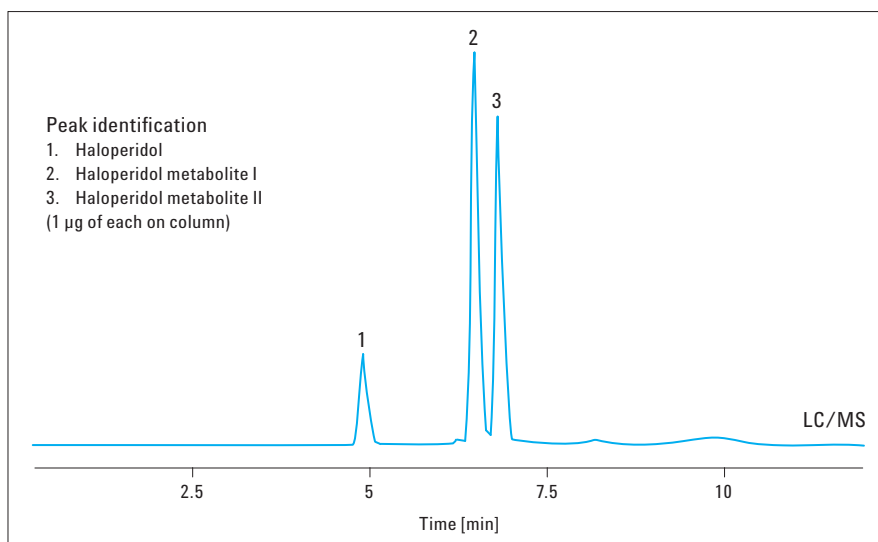
Column: C8, 50 × 2.1 mm, 5 µm
Eluent A: 0.1 % TFA in Water
Eluent B: 0.1 % TFA in Acetonitrile
Gradient: 5-95 % in 10 min
Flow rate: 0.2 mL/min
Injection volume: 10 µL
Detection: Agilent 385-ELSD (neb. = 60 °C, evap. = 30 °C, gas flow = 1.3 SLM)

Monitoring pharmaceutical libraries using LC/MS and ELS detection

Couple the Agilent 385-ELSD with LC/MS to reveal much greater detail in the analysis of haloperidol and its metabolites

The structural information obtained from an LC/MS system is extremely useful for monitoring pharmaceutical libraries. However, for compounds that do not 'fly' well on mass spectrometers, such as haloperidol, the Agilent 385-ELSD will detect these molecules to provide complementary information and more accurate data. The 385-ELSD shows better sensitivity than LC/MS to haloperidol and its metabolites, demonstrating the power of ELS detection in pharmaceutical analysis.

Column: Agilent Pursuit XRs C18, 150 × 2.0 mm, 5 μm
 Eluent A: 0.1 % Formic acid in Water
 Eluent B: 0.1 % Formic acid in Acetonitrile
 Gradient: 10–50 in 7 min, 50–85 in 10 s, hold 50 s, 85–10 in 12 s
 Flow rate: 0.2 mL/min
 Injection volume: 10 μL
 Detection: Agilent 385-ELSD (neb. = 45 °C, evap. = 80 °C, gas flow = 0.9 SLM)



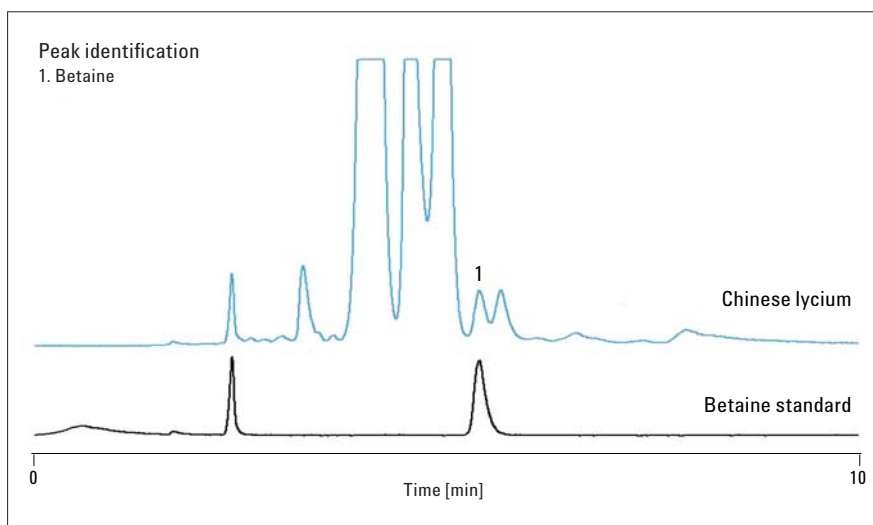
Peak description	MS detection (FS) S/N	ELS detection S/N	ELS detection sensitivity compared to MS detection
1. Haloperidol	1,348	37,400	28x
2. Metabolite I	2,084	68,000	33x
3. Metabolite II	1,046	13,000	12x

Analysis of nutraceuticals with ELS detection

Determining the level of betaine in Goji berries

Goji berries (or wolfberries) are a traditional Chinese medicine that contains high proportions of polysaccharides, vitamins C & B, amino acids, and betaine. Betaine maintains liver function and reduces levels of homocysteine. It has a weak UV chromophore, which limits its sensitivity by UV detection and prevents gradient elution on account of the low UV wavelength necessary for its detection. The universal response of the Agilent 385-ELSD provides better sensitivity to betaine than UV detection.

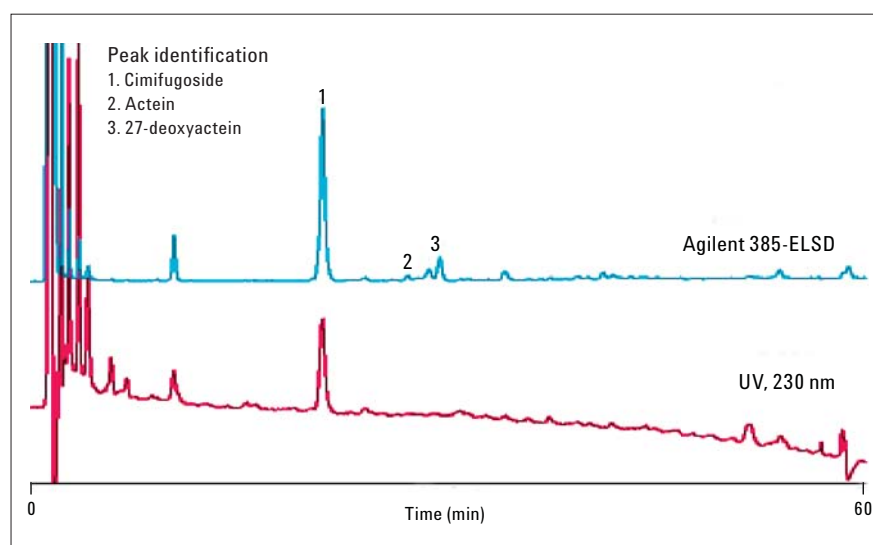
Column: HILIC, 250 × 4.6 mm, 5 μm
Eluent A: 10 mM Ammonium acetate, pH 6
Eluent B: Acetonitrile
Flow rate: 1.0 mL/min
Injection volume: 10 μL
Detection: Agilent 385-ELSD
(neb. = 30 °C, evap. = 40 °C, gas flow = 1.4 SLM)



Detection of triterpene glycosides in black cohosh

Triterpene glycosides possess weak or no UV chromophore, which limits their sensitivity by UV detection, especially during gradient elution at low wavelengths (for example, 230 nm). The Agilent 385-ELSD is the ideal detector for these compounds as it provides better sensitivity and baseline stability than UV detection.

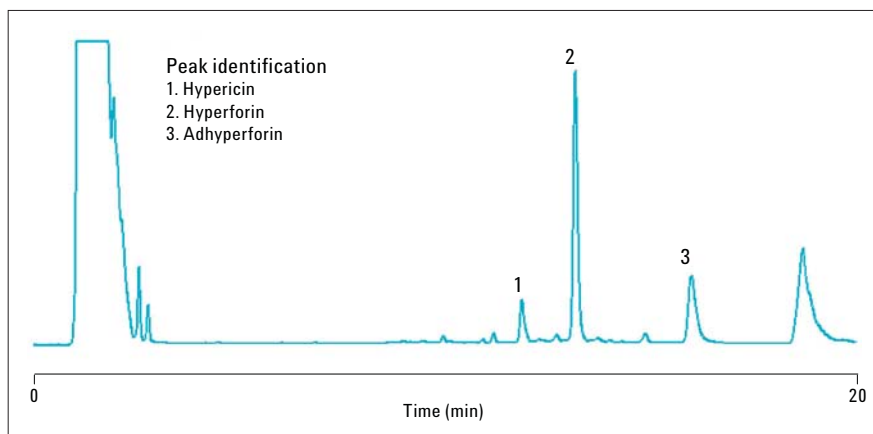
Column: C18, 150 × 4.6 mm, 5 μm
Eluent A: 0.1% Formic acid in water
Eluent B: Acetonitrile
Gradient: 30–40% B in 30 min, 40–60% B in 30 min, 60–30 % B in 10 min
Flow rate: 1.0 mL/min
Injection volume: 20 μL
Detection: Agilent 385-ELSD
(neb. = 30 °C, evap. = 50 °C, gas flow = 1.4 SLM)
UV detection: 230 nm



Analysis of hypericin in St John's Wort

The efficacy of natural products, such as St John's Wort, is a result of a complex mixture of active compounds. The universal nature of the Agilent 385-ELSD gives better mass balance of the components within St John's Wort and can detect all of the components in a single chromatographic run.

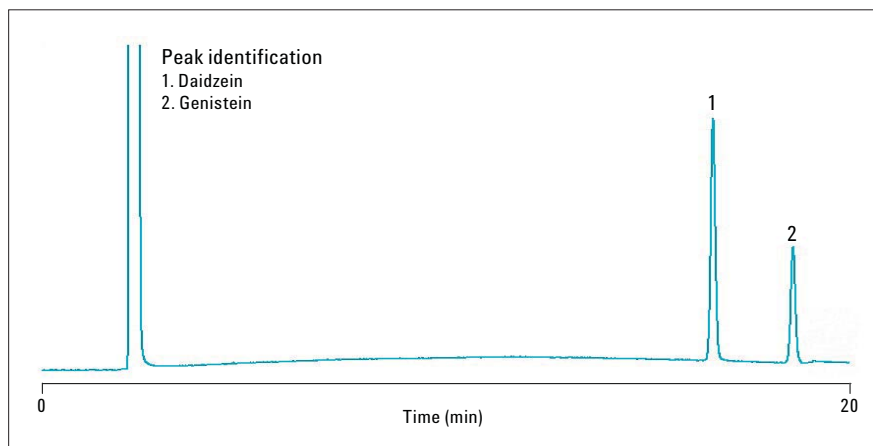
Column: C18, 150 × 4.6 mm, 5 μm
Eluent A: 0.1 % Ammonium formate, adjusted to pH 2.5
Eluent B: Acetonitrile
Gradient: 50–95% B in 10 min, hold 5 min
Flow rate: 1.0 mL/min
Injection volume: 20 μL
Detection: Agilent 385-ELSD
(neb. = 30 °C, evap. = 50 °C, gas flow = 1.6 SLM)



Determining the level of soflavones in soy protein

Isoflavones, such as genistein and daidzein, are found in soybeans and soy products like tofu. These compounds are of particular importance in reducing certain types of cancer. Genistein and daidzein show different UV activity, which creates a difference in their response for the same concentration. In turn, this leads to inaccurate mass balance data for mixtures of isoflavones. The Agilent 385-ELSD provides more accurate mass balance data compared to UV, which gives a better representation of isoflavones in complex soy mixtures.

Column: C18, 150 × 4.6 mm, 5 μm
Eluent A: 88/10/2 Water/MeOH/10% Acetic acid
Eluent B: 98/2 MeOH/10% Acetic Acid
Gradient: 20–30% B in 2 min, 30–50% B in 8 min, 50–70% in 6 min, 70–100 % in 0.1 min
Flow rate: 1.0 mL/min
Injection volume: 20 μL
Detection: Agilent 385-ELSD
(neb. = 30 °C, evap. = 30 °C, gas flow = 1.6 SLM)

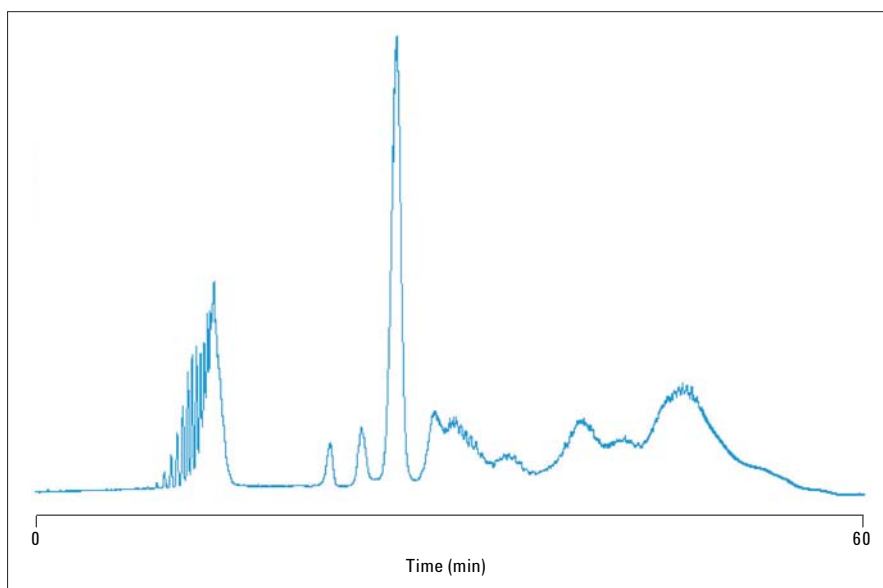


Analysis of excipients with ELS detection

Universal analysis of nonionic surfactants

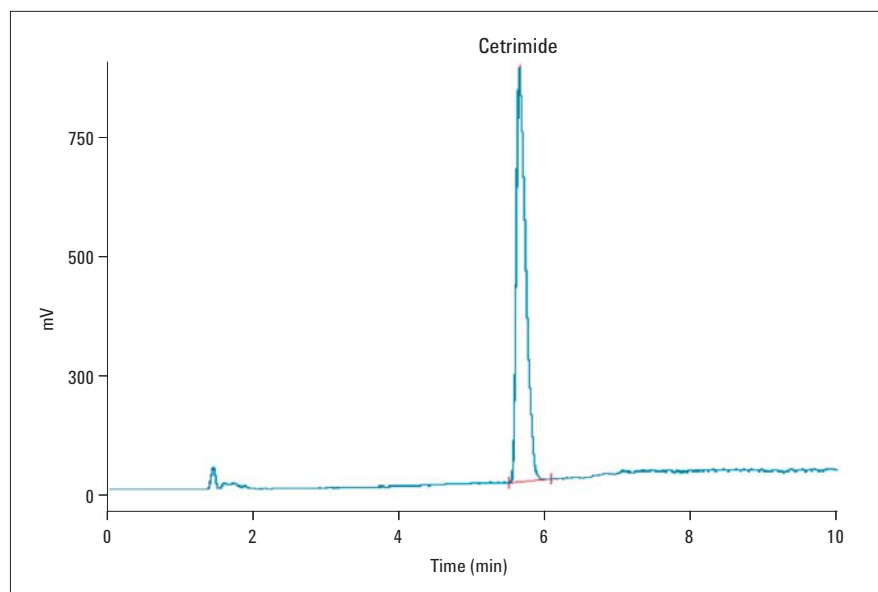
Tween 20, or polysorbate 20, is a non-ionic surfactant that effectively suppresses unspecific reactions between antibodies, antigens and other molecules. It is also used as a solubilizer in membrane chemistry and for density centrifugation of viruses. The absence of a usable UV chromophore and the requirement for gradient elution often mean that neither refractive index (RI) nor UV detection can be used. The Agilent 385-ELSD does not rely on the optical properties of a compound, making it the only choice for these types of excipients.

Sample: Tween 20 at 1 mg/mL
Column: PLRP-S 100 Å, 150 × 4.6 mm, 5 µm
Eluent A: Water
Eluent B: Acetonitrile
Gradient: 10–100% B in 55 min
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Detection: Agilent 385-ELSD (neb. = 30 °C, evap. = 50 °C, gas flow = 1.6 SLM)



Easy analysis of cationic surfactants

Cationic surfactants, such as cetrimide (alkyltrimethylammonium bromide), are typically used as antiseptic agents or detergents in fabric softeners, corrosion inhibitors and cosmetic products. Cetrimide is also a common ingredient of shampoos for treating seborrhoea and psoriasis. The 385-ELSD provides a quick and easy method for detecting these types of compounds without the need for chemical derivatization. ELS detection is more sensitive than RI detection providing lower detection levels while its gradient compatibility allows rapid separation of complex surfactant mixtures.

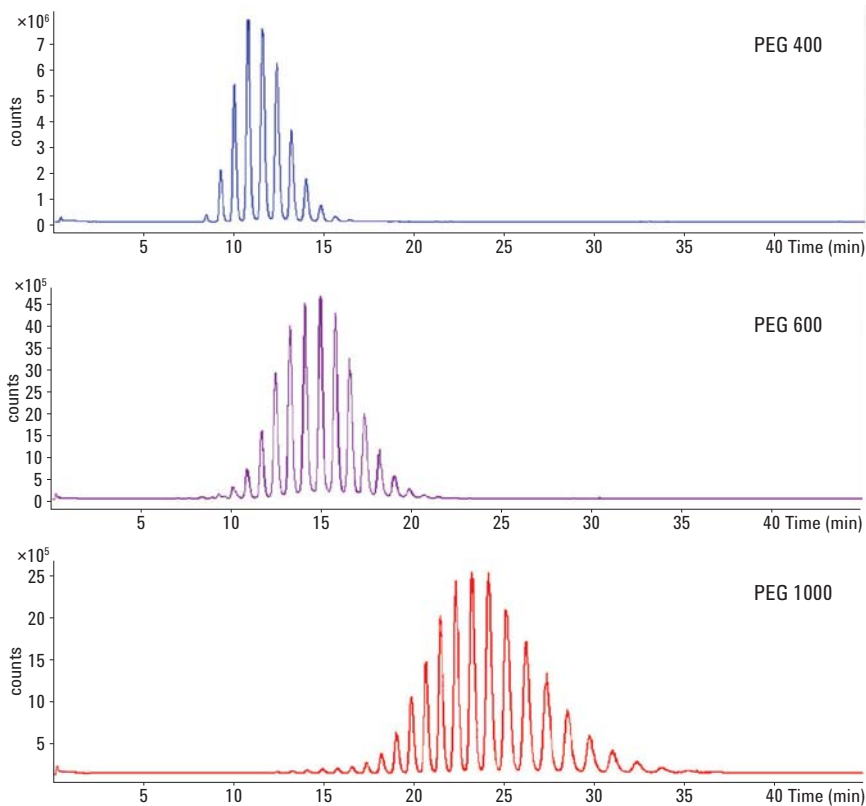


Sample: Cetrimide
Column: C18, 150 × 4.6 mm, 5 µm
Eluent A: 0.1% HFBA in Water
Eluent B: 0.1% HFBA in Acetonitrile
Gradient: 95:5 A:B
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Detection: Agilent 385-ELSD (neb. = 30 °C, evap. = 60 °C, gas flow = 1.4 SLM)

Determination of PEG using SFC/ELSD

Supercritical fluid chromatography (SFC) provides faster, higher resolution chromatography compared to HPLC. Consequently, SFC can increase throughput within a pharmaceutical or bioanalytical laboratory where a large number of potential drug compounds are screened.

ELS detection is a complementary detection technique for SFC because of the gaseous nature of the mobile phase. Using SFC/ELSD, evaporation of the eluent is not required as the mobile phase is volatilized during nebulization. Unlike flame ionization (FID), which is the common universal detector for SFC, the Agilent 385-ELSD is not affected by typical organic modifiers, such as methanol. Unlike most ELS detectors, the 385-ELSD comes with a heated nebulizer as standard, which is required for SFC operation, and so the 385-ELSD requires no modification for SFC use. The benefits of SFC/ELSD are highlighted with the high resolution separations of different polyethylene glycol samples.



www.agilent.com/chem/elsd

© Agilent Technologies, Inc., 2011-2015
Published in USA, August 1, 2015
5990-9455EN



Agilent Technologies