

Analysis of foods using HPLC with evaporative light scattering detection

Application Compendium

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Universal detection for food analysis

The Agilent 1290 Infinity 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 1290 Infinity 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 1290 Infinity 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 1290 Infinity ELSD is the evaporative light scattering detector of choice for food 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 non-volatile 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 1290 Infinity 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 1290 Infinity 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, especially important for work with small columns.

Optical Detection

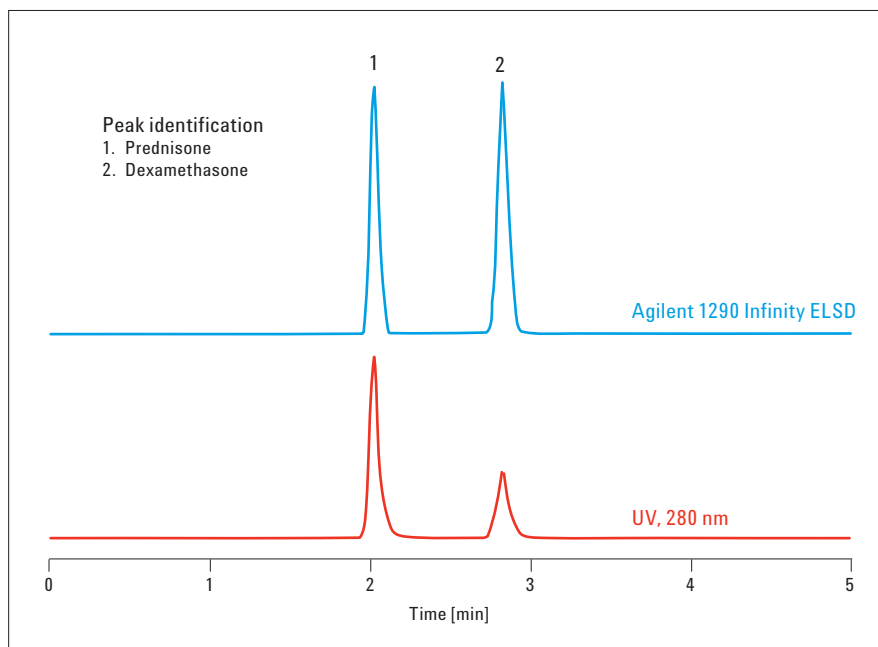
The solute particles are detected as they pass through the optical chamber. The high power LED and advanced design of the electronics deliver maximum sensitivity.

Benefits of ELS Detection

Obtain a more uniform response

The Agilent 1290 Infinity ELSD is not dependent on a compound's optical properties and 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.

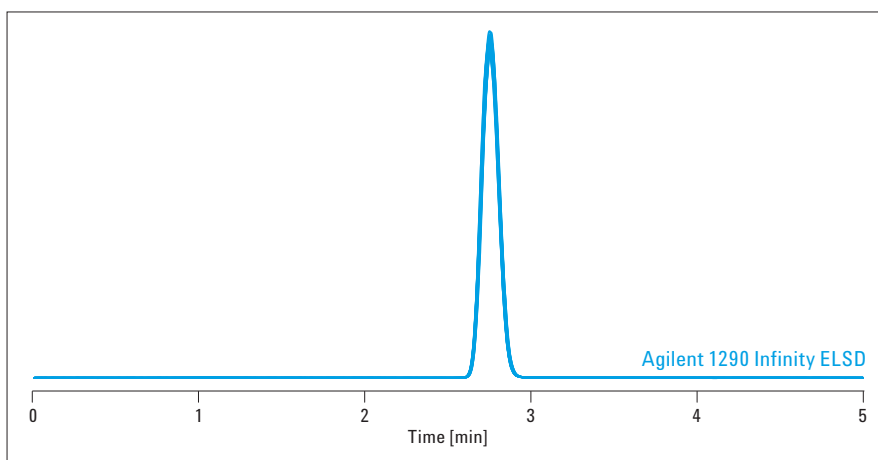
Column: Agilent Polaris C18,
150 × 4.6 mm, 5 μm
Eluent: Water:Acetonitrile 50:50
Flow rate: 1.0 mL/min
Injection volume: 10 μL
ELS detection: Agilent 1290 Infinity ELSD
(neb. = 30 °C, evap. = 30 °C,
gas flow = 1.4 SLM)
UV detection: 280 nm



Achieve superb RSD – 50 caffeine injections

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

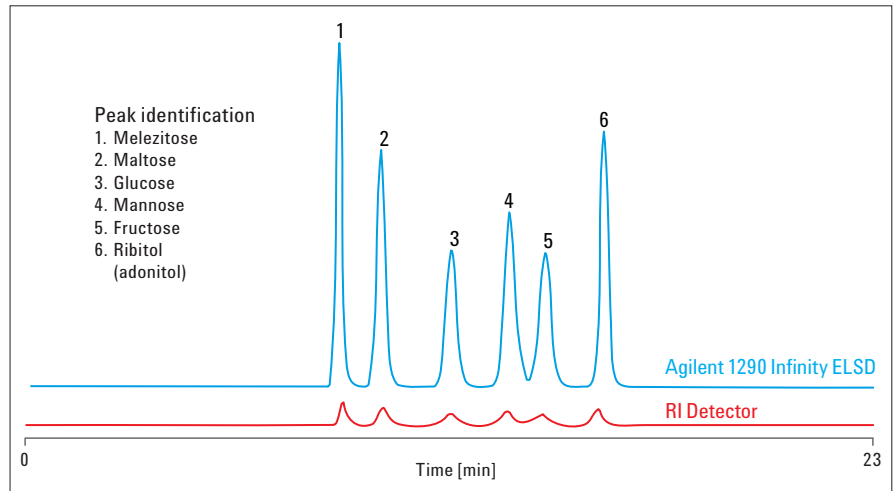
Column: Agilent Pursuit C18,
150 × 4.6 mm, 5 μm
Eluent: Water:Acetonitrile 40:60
Flow rate: 1.0 mL/min
Injection volume: 10 μL
ELS detection: Agilent 1290 Infinity ELSD
(neb. = 40 °C, evap. = 40 °C,
gas flow = 1.4 SLM)



Obtain higher sensitivity than with an RI detector

The Agilent 1290 Infinity ELSD has better baseline stability and sensitivity than an RI detector, making it an extremely suitable choice for carbohydrate analysis.

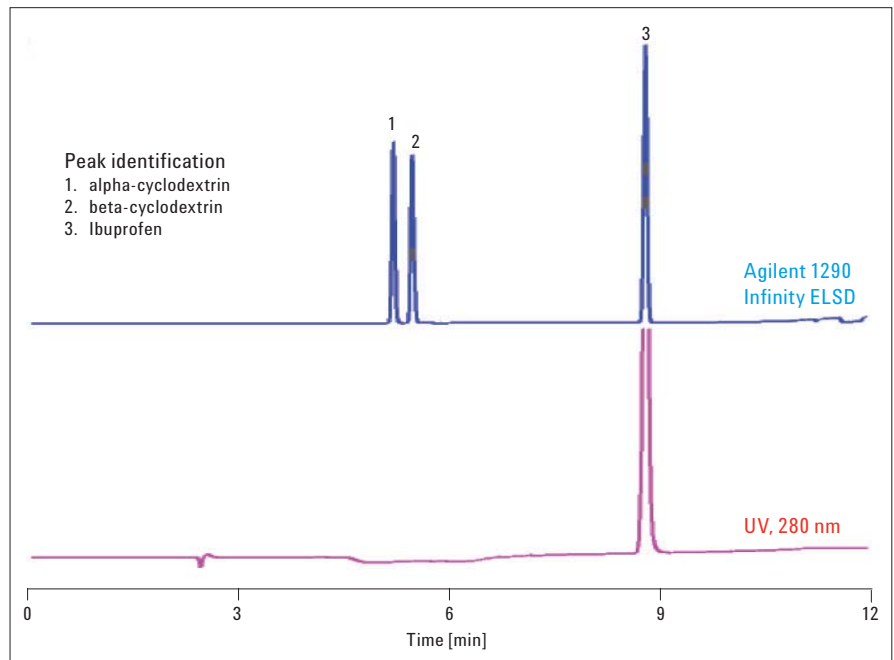
Sample: Carbohydrates
 Column: Hi-Plex Ca, 250 × 4 mm
 Eluent: Water
 Flow rate: 0.6 mL/min
 Temperature: 85 °C
 Injection volume: 10 µL
 Detection: Agilent 1290 Infinity ELSD
 (neb. = 30 °C, evap. = 30 °C,
 gas flow = 1.6 SLM)



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.

Sample: Tertiary aminols
 Column: C18, 150 × 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 1290 Infinity ELSD
 (neb. = 30 °C, evap. = 50 °C,
 gas flow = 1.0 SLM)

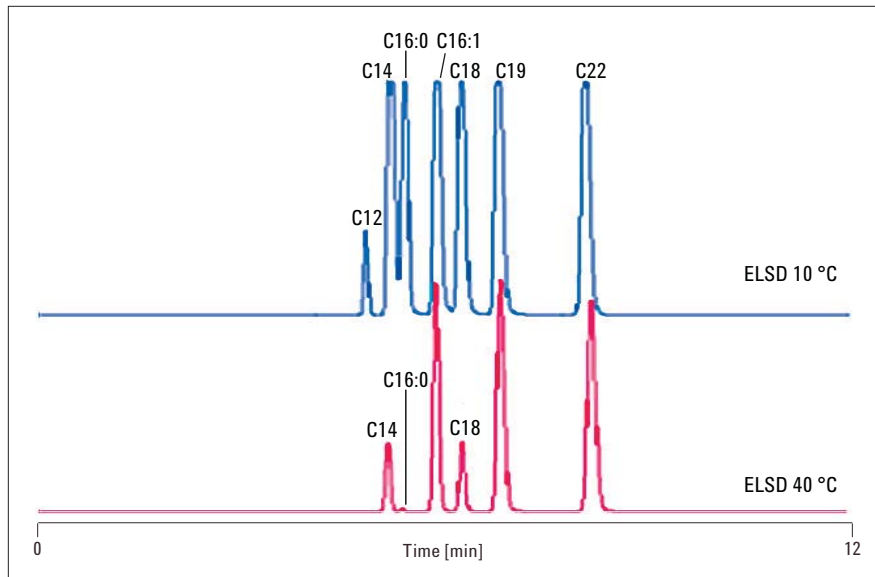


Lipids, fats, and oils

Determine FAME using ELS detection at 10 °C

Fatty acid methyl esters (FAME) are usually analyzed by GC/MS due to their high volatility. The FAMEs below C16 have typically been too volatile for ELSD, but the unique subambient operation of the Agilent 1290 Infinity ELSD allows FAMEs to be analyzed at 10 °C, extending the detection range to include lauric acid methyl ester (C12).

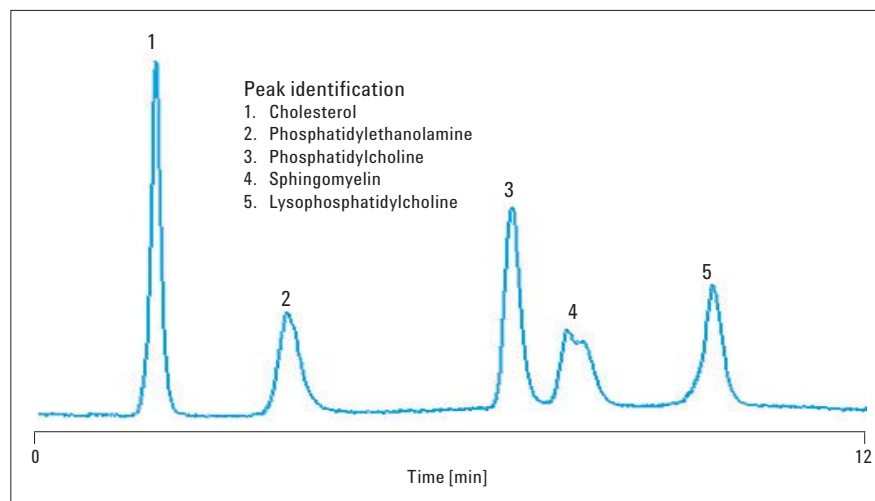
Sample: Fatty Acid Methyl Esters mix (C8 to C22)
 Column: C18, 250 × 4.0 mm, 5 µm
 Eluent A: Acetonitrile
 Eluent B: Dichloromethane
 Isocratic: 75/25 A/B
 Flow rate: 1.0 mL/min
 Injection volume: 5 µL
 Detection: Agilent 1290 Infinity ELSD (neb. = 80 °C, evap. as shown, gas flow = 1.6 SLM)



Detect phospholipids in complex matrices

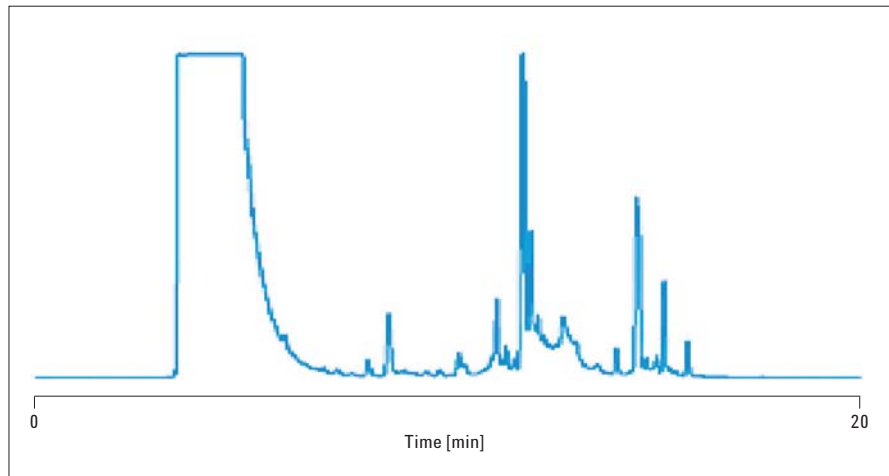
Most polar lipids exhibit very poor UV chromophores and are typically derivatized to enhance their absorbance in the UV range. The use of RI detection is also not possible because complex gradients are required to attain the necessary resolution of phospholipid mixtures. The Agilent 385-ELSD provides universal detection that obviates the need for derivatization, allowing the rapid determination of lipids in complex matrices.

Column: DIOL, 150 × 2.1 mm, 5 µm
 Eluent A: IPA/Hexane/Water/Ammonium Hydroxide 57.8/40/2/0.2
 Eluent B: IPA/Hexane/Water/Ammonium Hydroxide 51.8/40/8/0.2
 Gradient: 0–100% B in 7 min, 8 min hold, 100–0% B in 5 min, 10 min hold
 Flow rate: 0.3 mL/min
 Injection volume: 5 µL
 Detection: Agilent 1290 Infinity ELSD (neb. = 30 °C, evap. = 80 °C, gas flow = 1.0 SLM)



Detect compounds using complex gradient elution

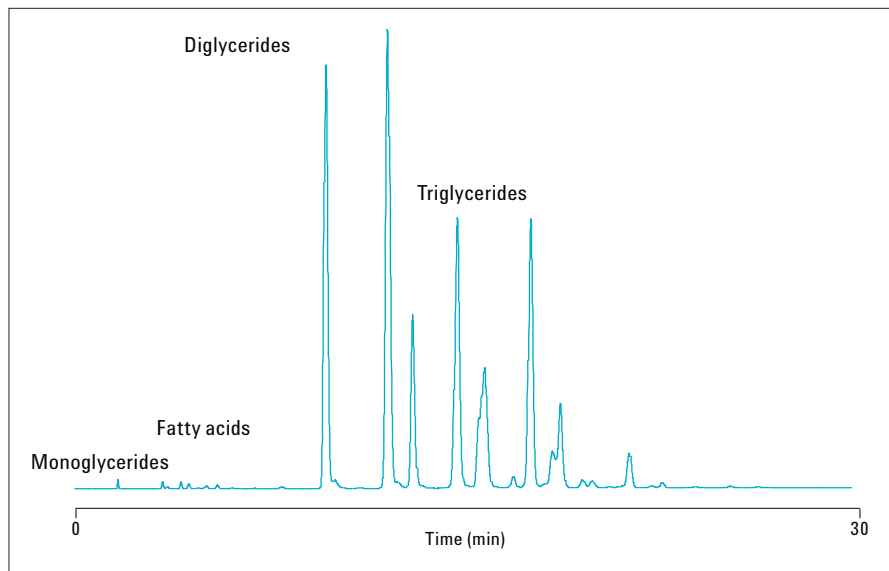
Biodiesels can be produced from almost any vegetable oil, such as sunflower and rapeseed. They contain complex mixtures of fatty acid methyl esters and lipids, all of which possess a weak or no UV chromophore. Consequently, fatty acids and lipids are often derivatized to enhance their UV absorbance or to facilitate their detection by LC/UV or GC/MS. ELS detection removes the need to derivatize fatty acids and lipids, thus increasing sample throughput. Unlike UV and RI detection, ELS detection is fully gradient compatible, which is a key advantage when analyzing biodiesels, where complex gradient elution is necessary to achieve the desired separation and resolution.



Sample:	Biodiesel	Gradient:	100% A, hold 5 min, 0–80% B in 10 min, hold 2 min, 80–0% B in 3 min
Column:	Cyano, 250 × 4.6 mm, 3 μm	Flow rate:	1.0 mL/min
Eluent A:	0.4% Acetic acid in Hexane	Injection volume:	20 μL
Eluent B:	0.4% Acetic acid in Methyl tert-Butyl Ether	Detection:	Agilent 1290 Infinity ELSD (neb. = 40 °C, evap. = 90 °C, gas flow = 0.8 SLM)

Achieve high sensitivity in triglycerides analysis

The composition of triglycerides in refined sesame oil using ELS detection is outlined in Ph. Eur. monograph 0433. The oil contains many chemically similar species and so gradient elution involving solvents with very different properties is required to separate the components. For this reason, RI is not a viable method, while baseline disturbances can arise with UV or limit the choice of solvent. The Agilent 1290 Infinity ELSD is unaffected by the optical properties of the solvents and delivers high sensitivity.

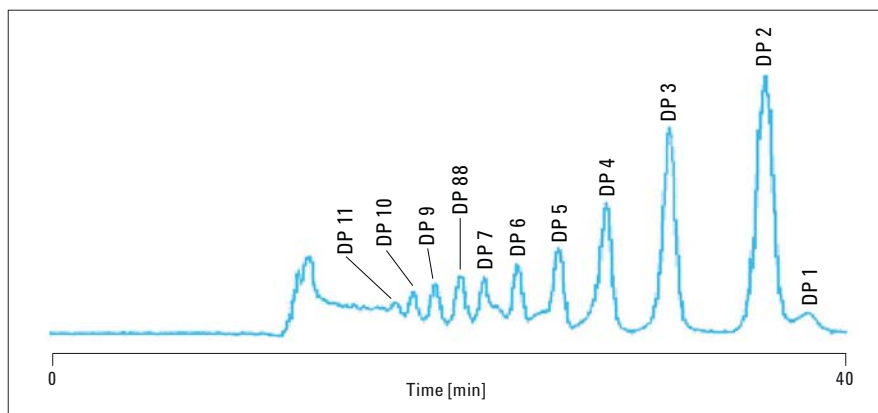


Sample:	Sesame oil	Gradient:	25% B hold 5 min, 25–50% B in 30 min, 50–90% B in 5 min
Column:	C18, 250 × 4.0 mm, 5 μm	Flow rate:	5 μL
Eluent A:	Acetonitrile	Detection:	Agilent 1290 Infinity ELSD (neb. = 60 °C, evap. = 50 °C, gas flow = 1.6 SLM)
Eluent B:	Dichloromethane		

Carbohydrates

Achieve high sensitivity and stable baselines

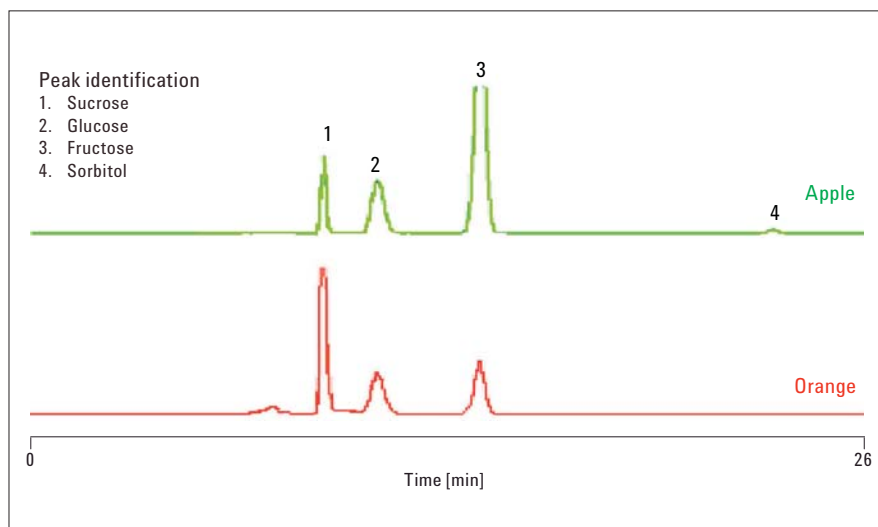
Corn syrup is a common additive in food due to its thickening and humectant properties that keep foods moist and help to maintain freshness. It is also used to soften texture, add volume and prevent crystallization. The oligosaccharide composition of corn syrup, with its different degrees of polymerization (Dp), can affect sucrose crystallization in foods. The Agilent 1290 Infinity ELSD is the ideal choice for determining the oligosaccharide composition of corn syrup, due to its high sensitivity and stable baseline, when compared to RI detection.



Sample:	Light corn syrup (Malto Oligosaccharides, Dp 1-Dp 9)	Injection volume:	10 μ L
Column:	Hi-Plex Na, 300 \times 7.7 mm	Temperature:	75 $^{\circ}$ C
Eluent:	Water	Detection:	Agilent 1290 Infinity ELSD (neb. = 50 $^{\circ}$ C, evap. = 70 $^{\circ}$ C, gas flow = 1.2 SLM)
Flow rate:	0.3 mL/min		

Improved detection of sugars in commercial fruit juices

The predominant sugars in natural fruit juice are sucrose, glucose, and fructose. The ratio of these three sugars differs between fruit but with each single fruit the ratio is relatively constant. Fruit juices derived from concentrate are often adulterated with sweeteners, water, or other types of fruit juices to extend the product life or to increase profit. The Agilent 1290 Infinity ELSD is universal and can be used to directly measure the ratio of sugars in fruit juices. Compared to RI detection, the 1290 Infinity ELSD provides a stable baseline, which improves the precision of the quantification.

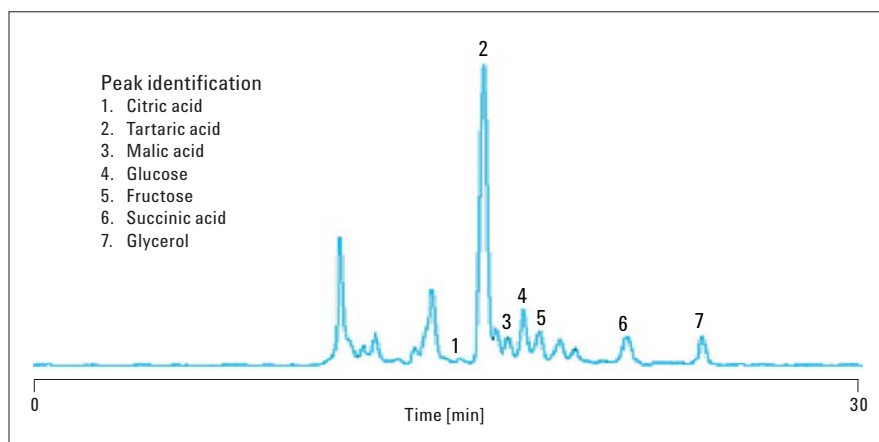


Sample:	Apple and orange juice	Injection volume:	5 μ L
Column:	Hi-Plex Ca, 300 \times 7.7 mm	Temperature:	80 $^{\circ}$ C
Eluent:	Water	Detection:	Agilent 1290 Infinity ELSD (neb. = 50 $^{\circ}$ C, evap. = 50 $^{\circ}$ C, gas flow = 1.2 SLM)
Flow rate:	0.6 mL/min		

Measure organic acids and sugars in red wine accurately

Organic acids play an important role in the fermentation of wine. The precise balance of organic acids and sugars must be achieved in order to obtain the correct composition, stability, and taste of the wine. The Agilent 1290 Infinity ELSD is a universal and uniform detector that provides a simple and direct method of measuring the relative composition of organic acids and sugars in wine.

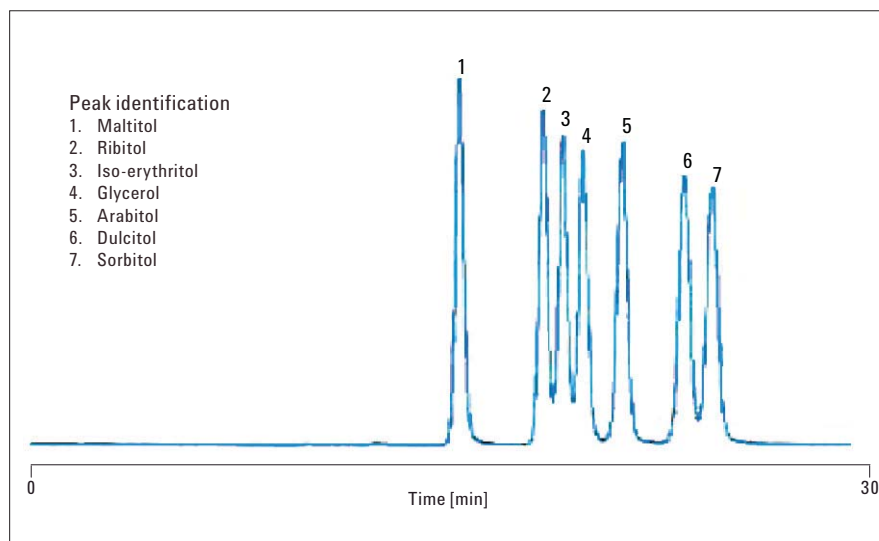
Sample: Australian Shiraz
Column: Hi-Plex H, 250 × 7.7 mm
Eluent: 0.1% TFA in Water
Flow rate: 0.4 mL/min
Injection volume: 2 µL
Temperature: 85 °C
Detection: Agilent 1290 Infinity ELSD
(neb. = 30 °C, evap. = 50 °C, gas flow = 1.2 SLM)



Detect sugar alcohols universally

Sugar alcohols are commonly used in place of sucrose, especially in diabetic foodstuffs. They also possess fewer calories than sucrose and do not contribute to tooth decay. The control of these additives in food can be carefully monitored using the 1290 Infinity ELSD.

Sample: Sugar alcohols
Column: Hi-Plex Ca, 250 × 4.0 mm
Eluent: Water
Flow rate: 0.6 mL/min
Injection volume: 10 µL
Temperature: 85 °C
Detection: Agilent 1290 Infinity ELSD
(neb. = 30 °C, evap. = 30 °C, gas flow = 1.6 SLM)

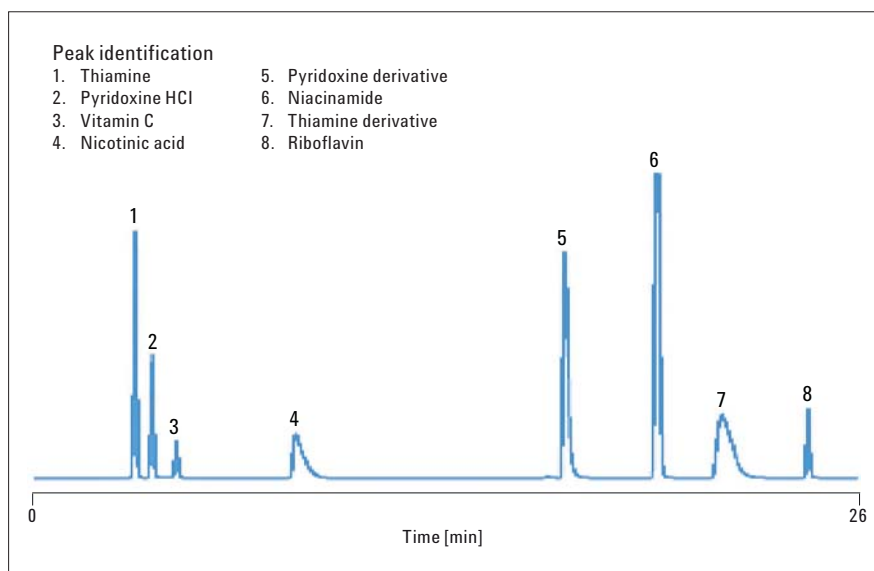


Vitamins and Food Dyes

Analyze accurate compositional data for vitamin mixtures

The universal and uniform response of the Agilent 1290 Infinity ELSD provides accurate compositional data for vitamin mixtures. Highly polar vitamins, such as thiamine and vitamin C, can be problematic for UV detectors as they elute at the solvent front. The 1290 Infinity ELSD evaporates the mobile phase before detection so does not exhibit any solvent front, which ensures that early eluting peaks are detected.

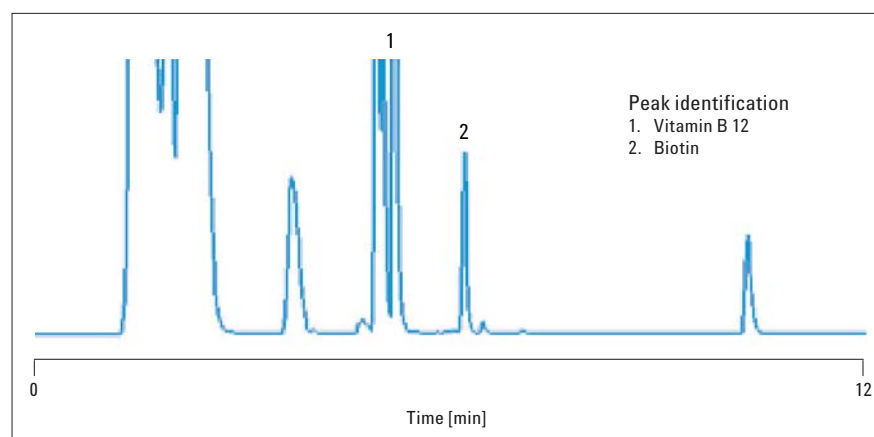
Sample: Water Soluble Vitamins mix
Column: C18, 150 × 4.6 mm, 5 μm
Eluent A: Water
Eluent B: Acetonitrile
Gradient: 100% A in 0–4 min,
0–50% B in 10 min
Flow rate: 0.6 mL/min
Injection volume: 10 μL
Detection: Agilent 1290 Infinity ELSD
(neb. = 25 °C, evap. = 25 °C,
gas flow = 1.6 SLM)



Quantify vitamin B12 and biotin in nutritional supplements

The Agilent 1290 Infinity ELSD provides direct quantification of biotin and B12 without the need for derivatization. The universal response and high sensitivity of the ELS detector allows biotin and vitamin B12 to be quantified in nutritional supplements.

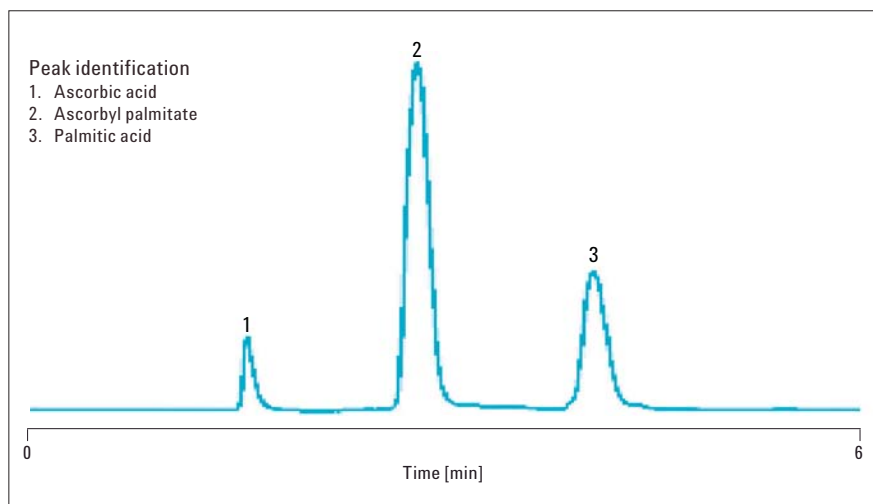
Sample: Nutritional supplements
Column: PLRP-S, 50 × 4.6 mm, 3 μm
100 Å
Eluent A: 0.1% TFA in water
Eluent B: 0.1% TFA in ACN
Gradient: 1–99% B in 10 min
Flow rate: 0.5 mL/min
Injection volume: 100 μL
Detection: Agilent 1290 Infinity ELSD
(neb. = 25 °C, evap. = 25 °C,
gas flow = 1.6 SLM)



Assess ascorbic acid and derivatives with accuracy

Ascorbyl palmitate is an ester formed from ascorbic acid and palmitic acid creating a fat-soluble form of vitamin C. It is commonly used as an antioxidant food additive (E304). The Agilent 1290 Infinity ELSD can be used to accurately assess the relative ratios of these compounds due to its universal and uniform response.

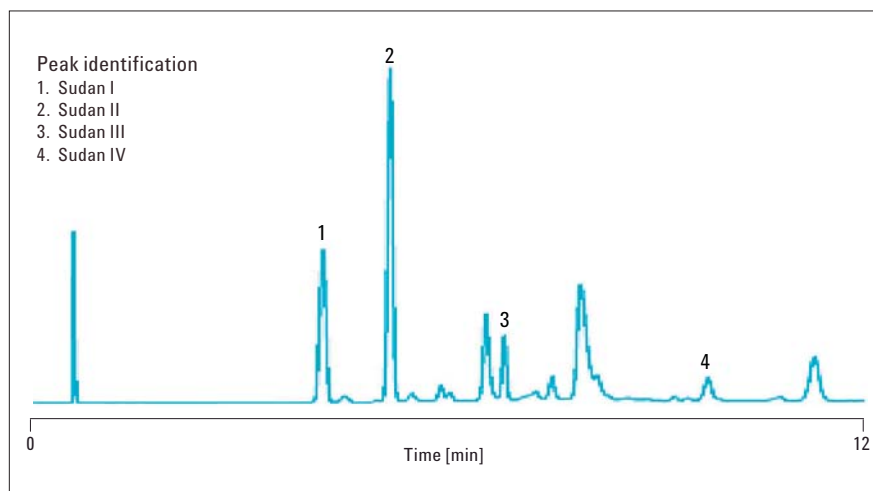
Column: C18, 150 × 4.6 mm, 5 μm
Eluent A: 1% Formic acid in Water
Eluent B: Acetonitrile
Eluent C: Methanol
Isocratic: 2.5/50/47.5 A/B/C
Flow rate: 1.5 mL/min
Injection volume: 10 μL
Detection: Agilent 1290 Infinity ELSD
(neb. = 30 °C, evap. = 30 °C,
gas flow = 1.4 SLM)



Determine Sudan dyes in paprika oil

The Agilent 1290 Infinity ELSD is universal and is not dependent on the optical properties of the compound. Consequently, for food containing Sudan dyes (I, II, III and IV) that possess UV different extinction coefficients, the ELS detector delivers a more uniform and representative response.

Sample: Paprika Oleoresin
Column: C18, 150 × 2.1 mm, 5 μm
Eluent A: 0.1% Acetic acid in water
Eluent B: 0.1% Acetic acid in ACN
Gradient: 70–95% B in 10 min,
hold 10 min
Flow rate: 0.23 mL/min
Injection volume: 20 μL
Detection: Agilent 1290 Infinity ELSD
(neb. = 30 °C, evap. = 30 °C,
gas flow = 1.6 SLM)



Sensitive detection of lipids through sub-ambient evaporation and nebulization

The Agilent 1290 Infinity ELSD is equipped with a Peltier cooled evaporation tube which enables solvent removal at sub-ambient temperatures. This is an interesting feature for the detection of (semi-)volatile compounds. A standard mixture of lipids comprising 500 µg/mL 1-monopalmitin (MP), 1,2-dipalmitin (DP), tripalmitin (TP), and palmitic acid methyl ester (PAME) was used to demonstrate that all components can be detected at sub-ambient conditions with the evaporator temperature at 15 °C and the nebulizer at 25 °C.

Column: Agilent ZORBAX Eclipse XDB C18 RRHT, 2.1 mm L × 50 mm id, 1.8 µm

Eluent A: 0.1% acetic acid in methanol

Eluent B: Isopropanol/hexane 50/40 v/v

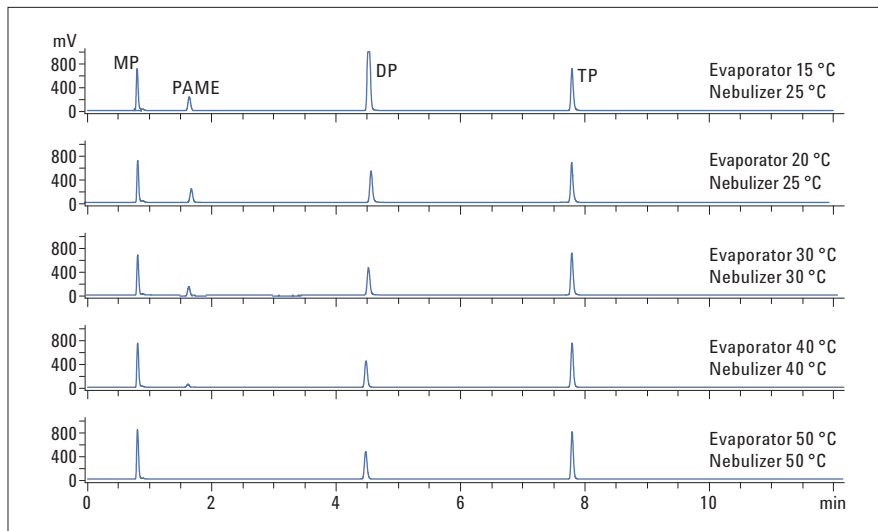
Flow rate: 1 mL/min

Gradient: 0% B from 0–1 min, 70% B at 11 min, hold 70% B to 12 min, 0% B at 14 min (post time)

Column Temperature: 25 °C

Injection volume: 2.5 µL, needle wash (4 s, flushport, mobile phase B)

Detection: Agilent 1290 Infinity ELSD (neb. varied 25–60 °C, evap. varied 15–60 °C, gas flow = 1.6 SLM, data rate = 40 Hz, smoothing = 3.0 s, gain = 1)



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© Agilent Technologies, Inc., 2011-2015
Published in USA, August 1, 2015
5990-9456EN



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