# Evaporative Light Scattering Detectors from Polymer Laboratories

# Introduction

- Polymer Laboratories has over 15 years of experience in ELSD
- ELSD can outperform traditional detectors when analysing non-chromophoric samples by HPLC
- Traditional HPLC detectors such as UV and RI have limited capabilities:
  - UV and RI are not compatible with a wide range of solvents
  - RI detection is not gradient compatible
  - Different analytes produce different UV responses depending on their extinction co-efficient
- ELSDs can detect anything that is less volatile than the mobile phase
- ELSD is universal and compatible with a wide range of solvents

# Introduction

Polymer Laboratories presents 3 models of ELSD, each offering high sensitivity for a wide range of applications:

**PL-ELS 2100**

- Regular HPLC with low temperature operation for volatile compounds

**PL-ELS 1000**

- Routine HPLC and GPC using high boiling point solvents

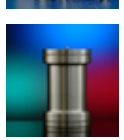
**PL-ELS 1000µ**

- For microbore and capillary LC









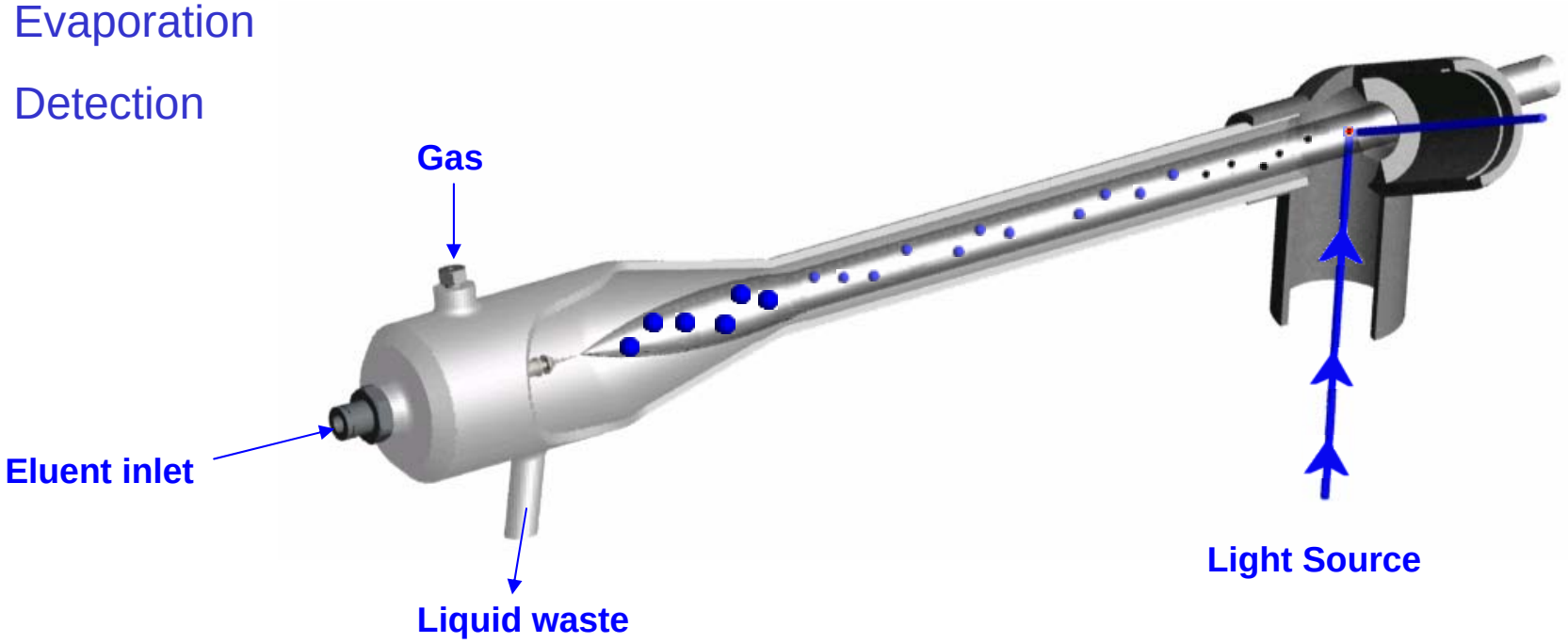


# New PL-ELS 2100 Detector

# Principles of Operation

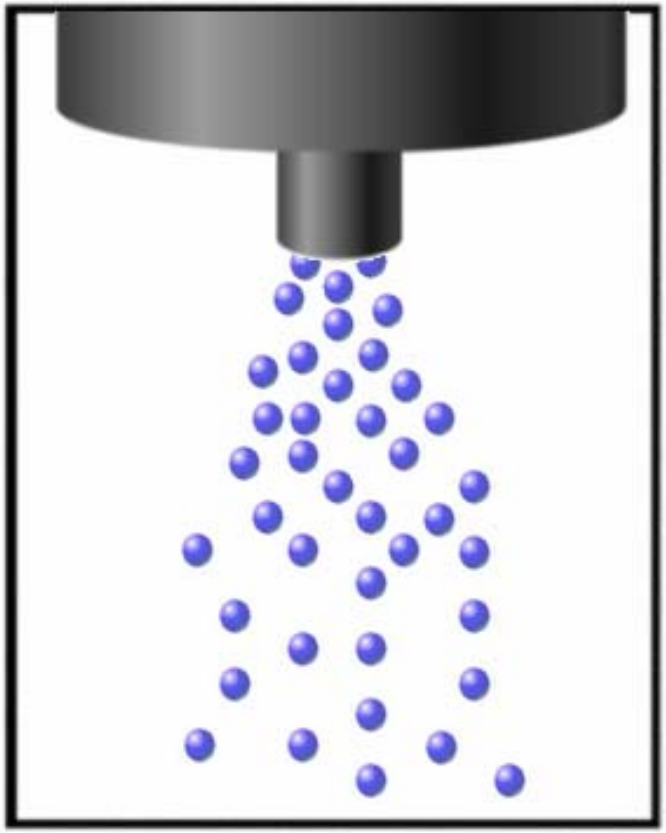
The ELSD principle of operation employs three distinct stages:

- Nebulisation
- Evaporation
- Detection



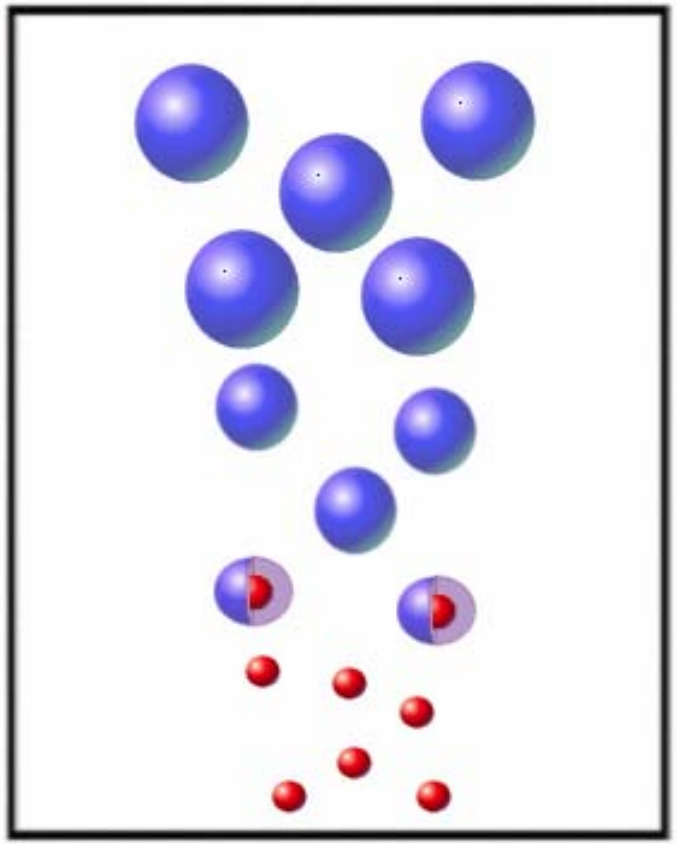
# Nebulisation

- Eluent flow mixed with N2 or Air
- Concentric nebuliser
- Efficient nebulisation: stable droplet plume, uniform droplet size
- Temperature independently controlled
  Narrower cone of the plume
  (minimise band broadening)
- Small nebuliser chamber
  (reduces band broadening)



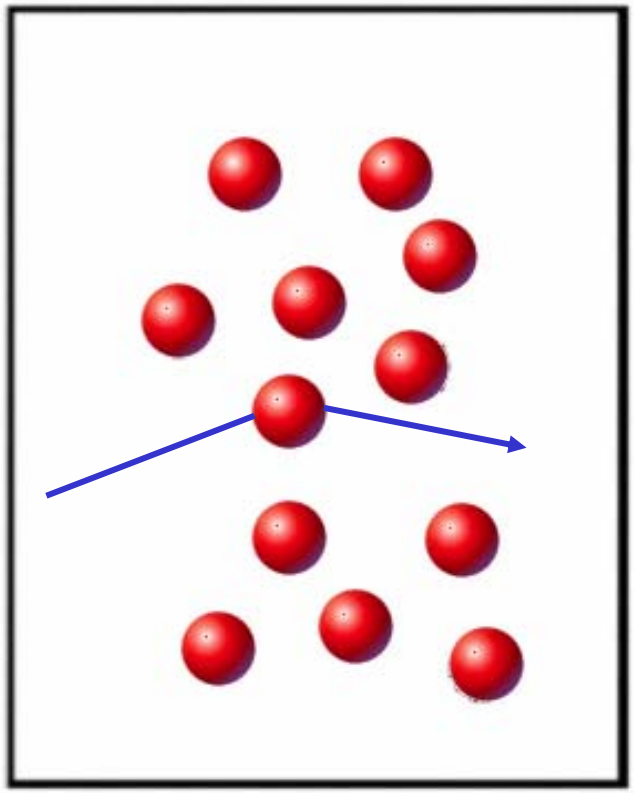
# Evaporation

- Droplets pass through heated drift tube
  - 30cm straight tube
- Removes mobile phase to leave particulate form of analyte
- Temperature set according to analyte volatility
- Temperature controlled by user
- Important to have laminar flow (reduces band broadening)



# Detection

- Particles irradiated with light source
  - LED (ca 400-500 nm)
- Particles scatter light according to their size (mass sensitive)
- Scattered light is detected by photomultiplier at fixed angle from incident light
- Detection independent of optical properties of analyte



# Advantages of Evaporative Light Scattering Detection

- Universal - responds to all compounds in the mobile phase
- Not dependent on spectroscopic properties of analyte
- Produces more uniform detection sensitivity for analytes
- Not susceptible to baseline drift during gradient elution, temperature or solvent pump fluctuations
- ELSD compatible with a much wider range of solvents compared to Refractive Index

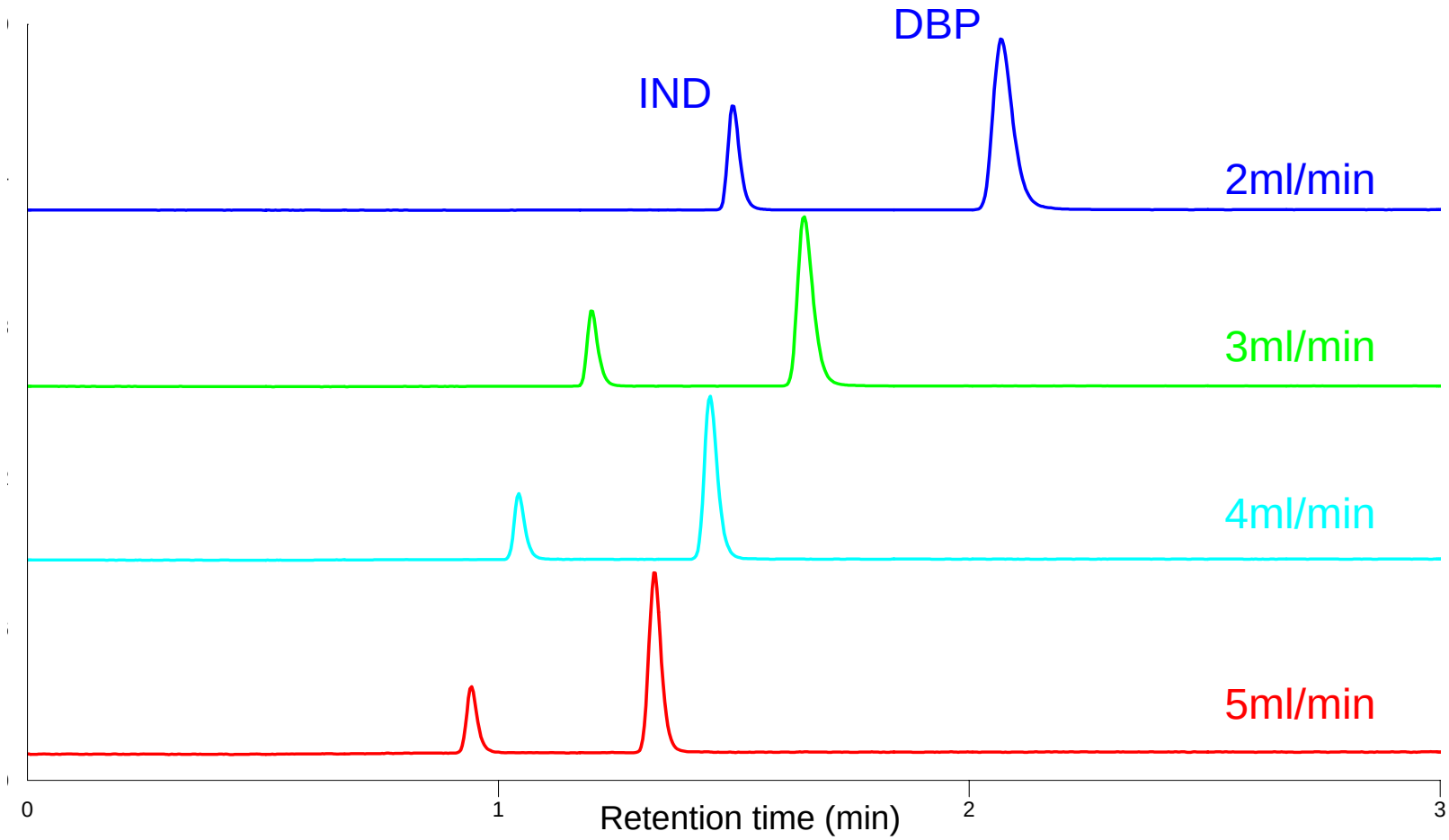
# Advantages of Evaporative Light Scattering Detection

- Removes the need for derivatisation steps (eg amino acids, toxins)
- Fast setup and equilibration
- Sensitivity in the order of 1-50ng **(on column)**
  (depends on eluent flow rate)
- No interference from solvent front peaks
  (enables fast analysis)
- Removal of mobile phase eluent allows rapid HPLC gradients
- Flow rates up to up to 5ml/min can be achieved with no affect on baseline stability
- Ideal for High Throughput Screening

# Fast Gradient, Fast Flow Rate Capability

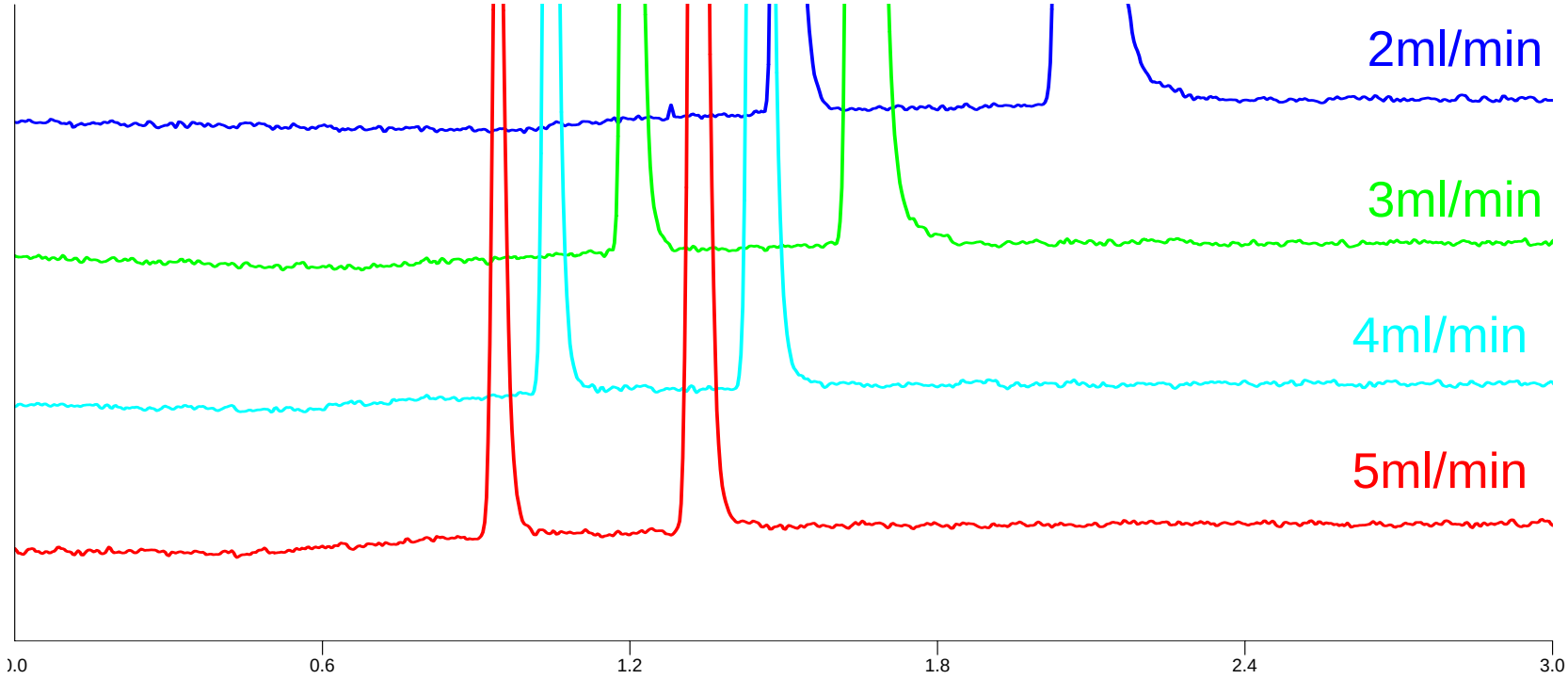
| | |
|---|---|
| Column: | PLRP-S 100Å 5µm, 50x4.6mm |
| Eluent A: | Water + 0.05% TFA |
| Eluent B: | ACN + 0.05% TFA |
| Gradient: | 5-95% B in 1 min |
| Detector: | PL-ELS 2100 (neb=30°C, evap=30°C, gas=1.6 SLM) |
| Sample: | Indapamide(IND), Dibutyl phthalate (DBP) |
| | Flow rate increased from 2ml/min up to 5ml/min |
| Note: | IND is non-volatile, DBP is relatively volatile |

# Fast Gradient, Fast Flow Rate Capability



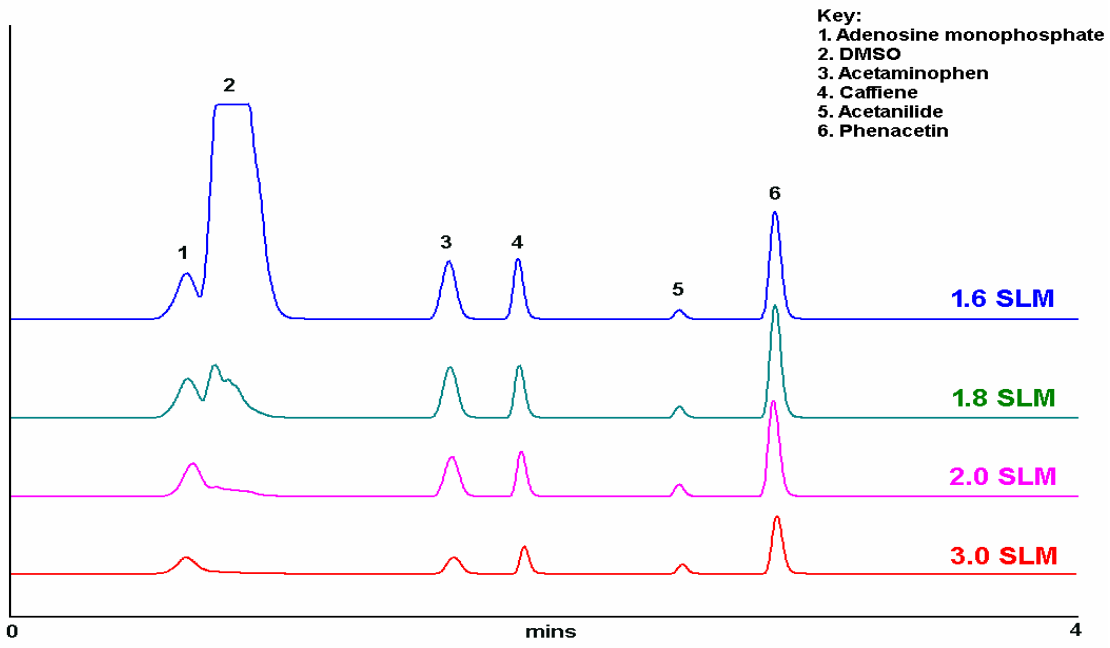
# Fast Gradient, Fast Flow Rate Capability

Stable baseline through the gradient



# High Throughput Screening

PL-ELS 2100 is transparent to DMSO at ambient temperature !!




**Nebuliser 30°C  Evaporator30°C**

**(peak 2 DMSO )**

# Evaporative Light Scattering Detection
# Ideal Complement to LC-MS

- Similar operating principles to LC-MS
  - Volatile buffers
  - Favours lower flow rates (ie 0.2-0.5ml/min)
- Can develop LC methods on ELSD then transfer to LC-MS
- ELSD can provide supporting information when used in tandem with LC-MS

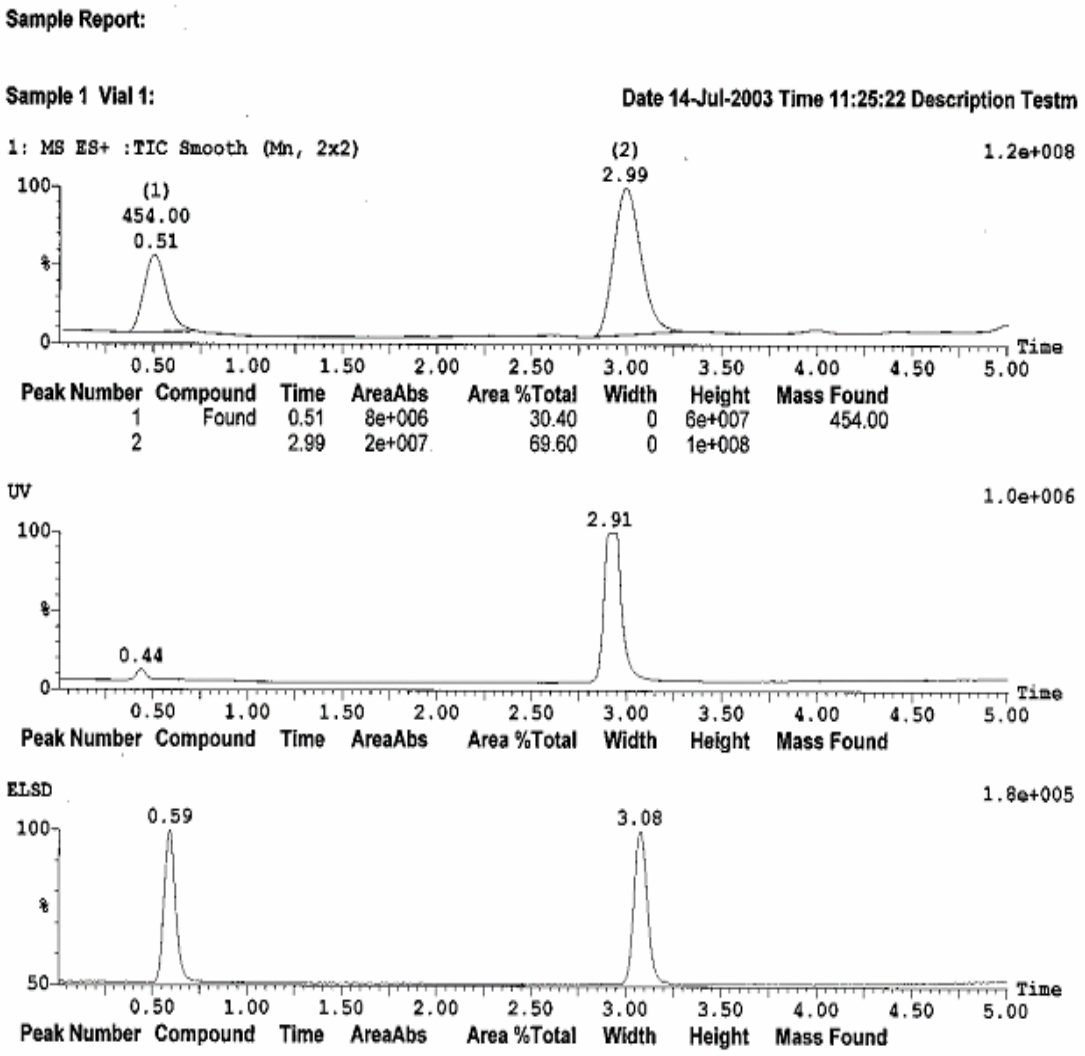
# Ideal Complement to LC-MS

**Sample Mixture of known 1:1 ratio**

**LC-MS**
**results show ratio to be**
**3:1**

**UV-Vis**
**results show ratio to be**
**10:1**

**PL-ELS 2100**
**results show ratio to be**
**1:1**
(Response independent
of optical properties)



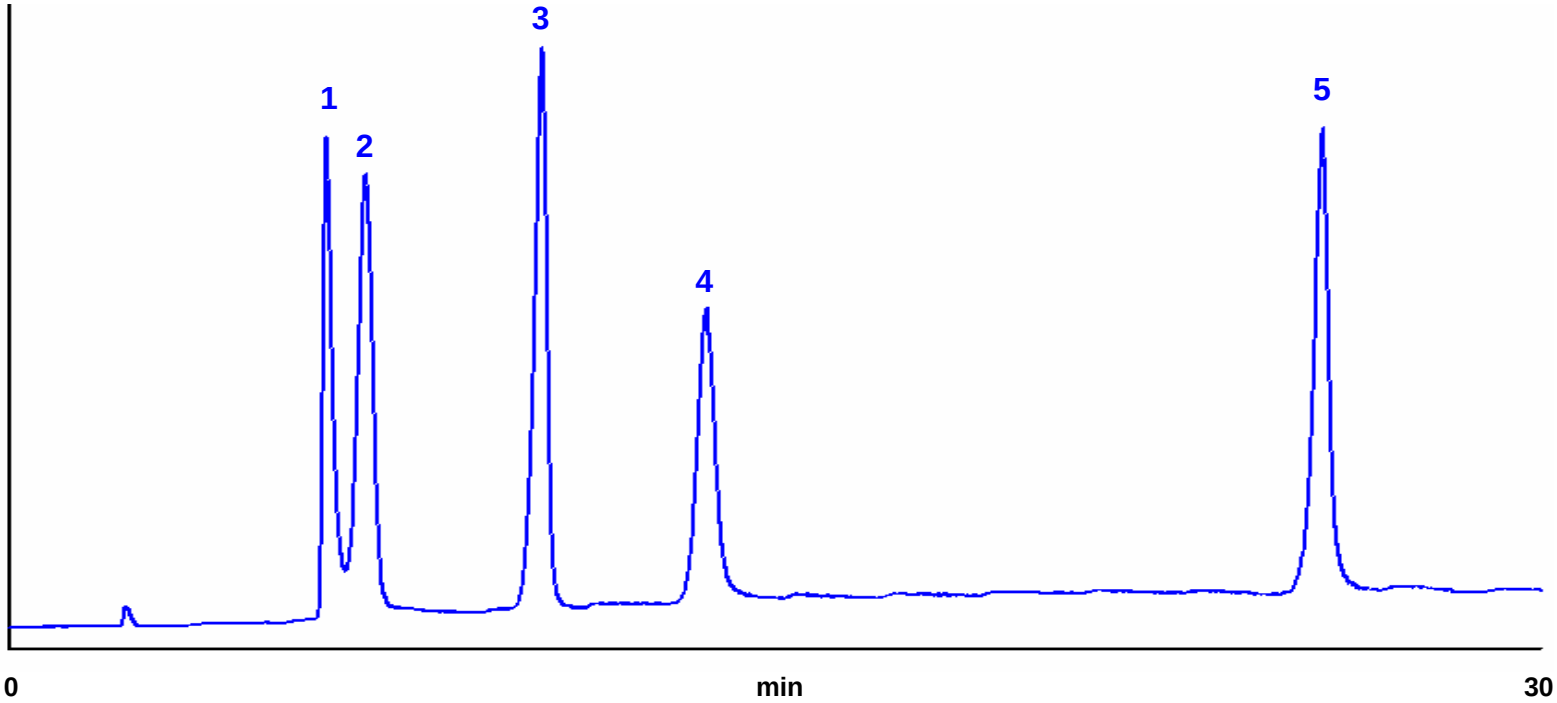
# Operation of the PL-ELS 2100

*For Volatile & Semi-Volatile Solutes*

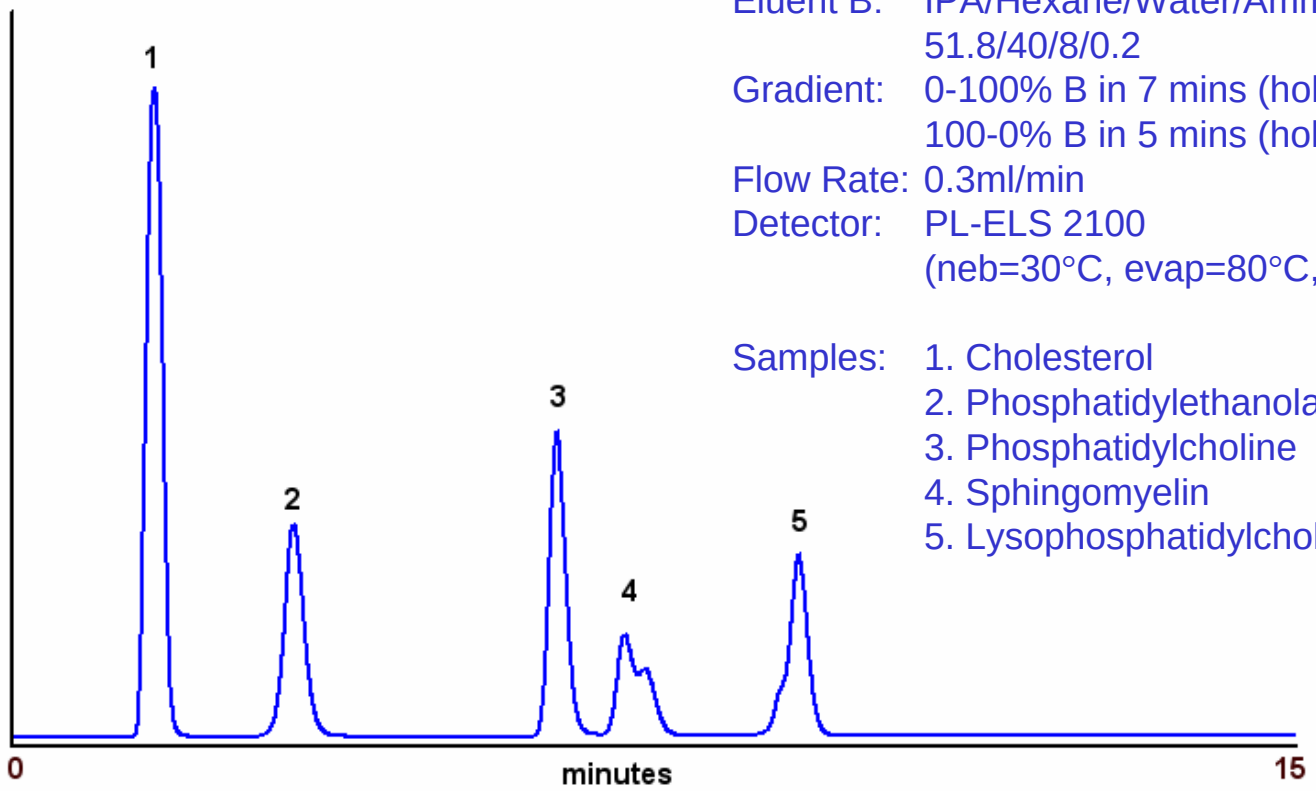
- A new approach to ELSD applications
- Nebuliser and evaporator temperatures determined by the volatility of the ANALYTE
- Gas flow used to aid evaporation of the mobile phase
  - 100% water @30°C requires high gas flow
  - 100% Hexane @30°C requires no gas flow
- PL-ELS 2100 can operate in 100% water @ 25°C

# Non-Volatile Application: Sugars

| | |
|---|---|
| Column: | PL Hi-Plex Ca 9µm, 300x7.7mm |
| Eluent: | Water |
| Flow Rate: | 0.6ml/min |
| Temp: | 85°C |
| Inj Vol: | 10µl |
| Detector: | PL-ELS 2100 (neb=30°C, evap=90°C, gas=1.6 SLM) |
| Samples: | 1. Fructose, 2. Glucose, 3. Sucrose I, 4. Lactose, 5. Stachyose |



# Non-Volatile Application: Phospholipid Separation




| | |
|---|---|
| Column: | Lichrospher DIOL 5µm,150x2.1mm |
| Eluent A: | IPA/Hexane/Water/Ammonia Hydroxide 57.8/40/2/0.2 |
| Eluent B: | IPA/Hexane/Water/Ammonia Hydroxide 51.8/40/8/0.2 |
| Gradient: | 0-100% B in 7 mins (hold 8 mins)<br>100-0% B in 5 mins (hold 10mins) |
| Flow Rate: | 0.3ml/min |
| Detector: | PL-ELS 2100<br>(neb=30°C, evap=80°C, gas=1.0 SLM) |

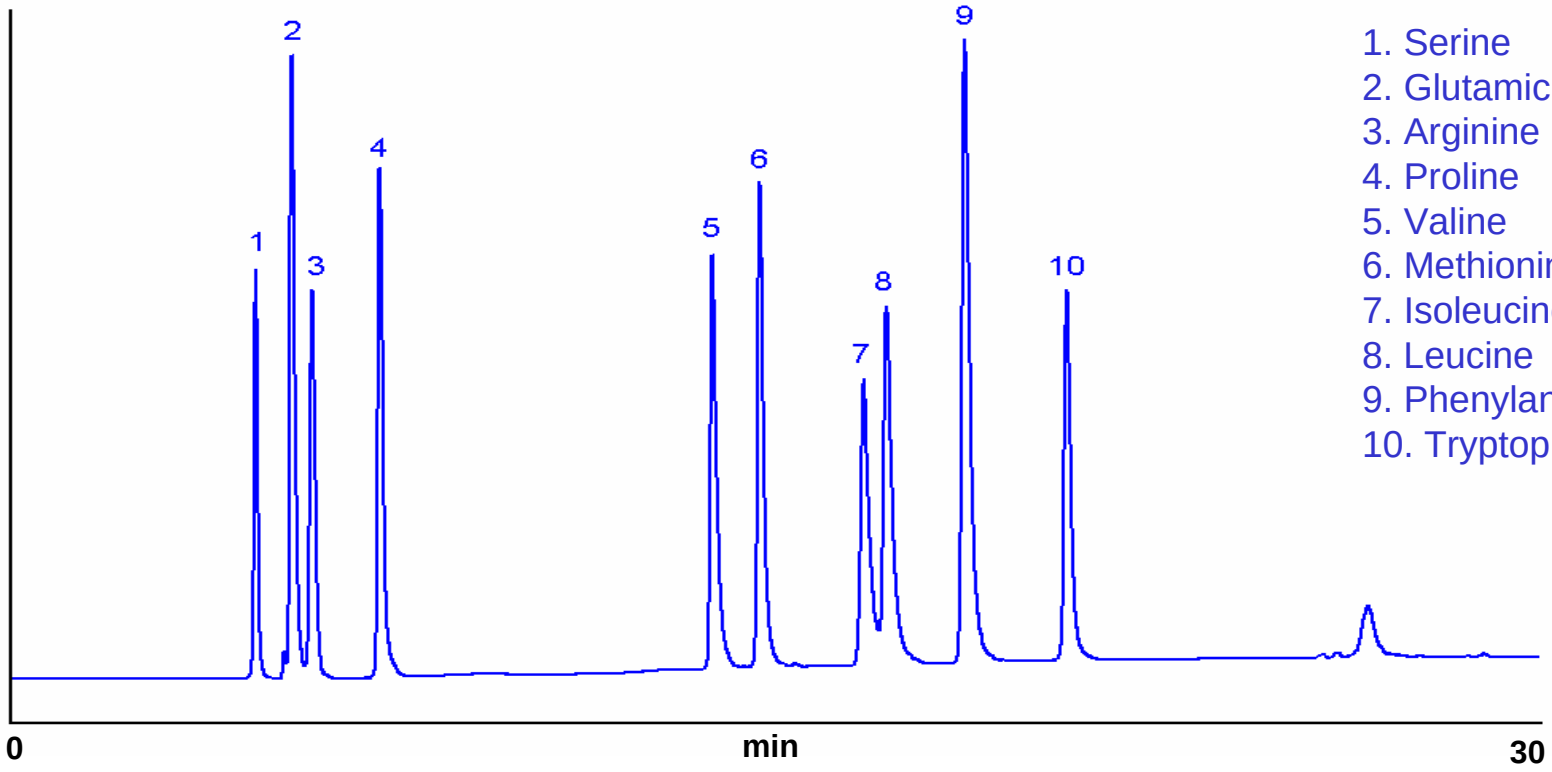
Samples:
1. Cholesterol
2. Phosphatidylethanolamine
3. Phosphatidylcholine
4. Sphingomyelin
5. Lysophosphatidylcholine

# Semi-Volatile Application: Underivatised Amino Acids

| | |
|---|---|
| Column: | Thermo-Hypersil ODS 5µm, 250x4.6mm |
| Eluent A: | Water |
| Eluent B: | Acetonitrile |
| Gradient: | 100% A in 5 mins hold, 0-40% B in 20 mins |
| Flow Rate: | 0.6ml/min |
| Inj Vol: | 10µl |
| Detector: | PL-ELS 2100 (neb=50°C, evap=50°C, gas=1.6 SLM) |

# Semi-Volatile Application: Underivatised Amino Acids

**ELSD removes the need for derivatisation for applications such as amino acids**



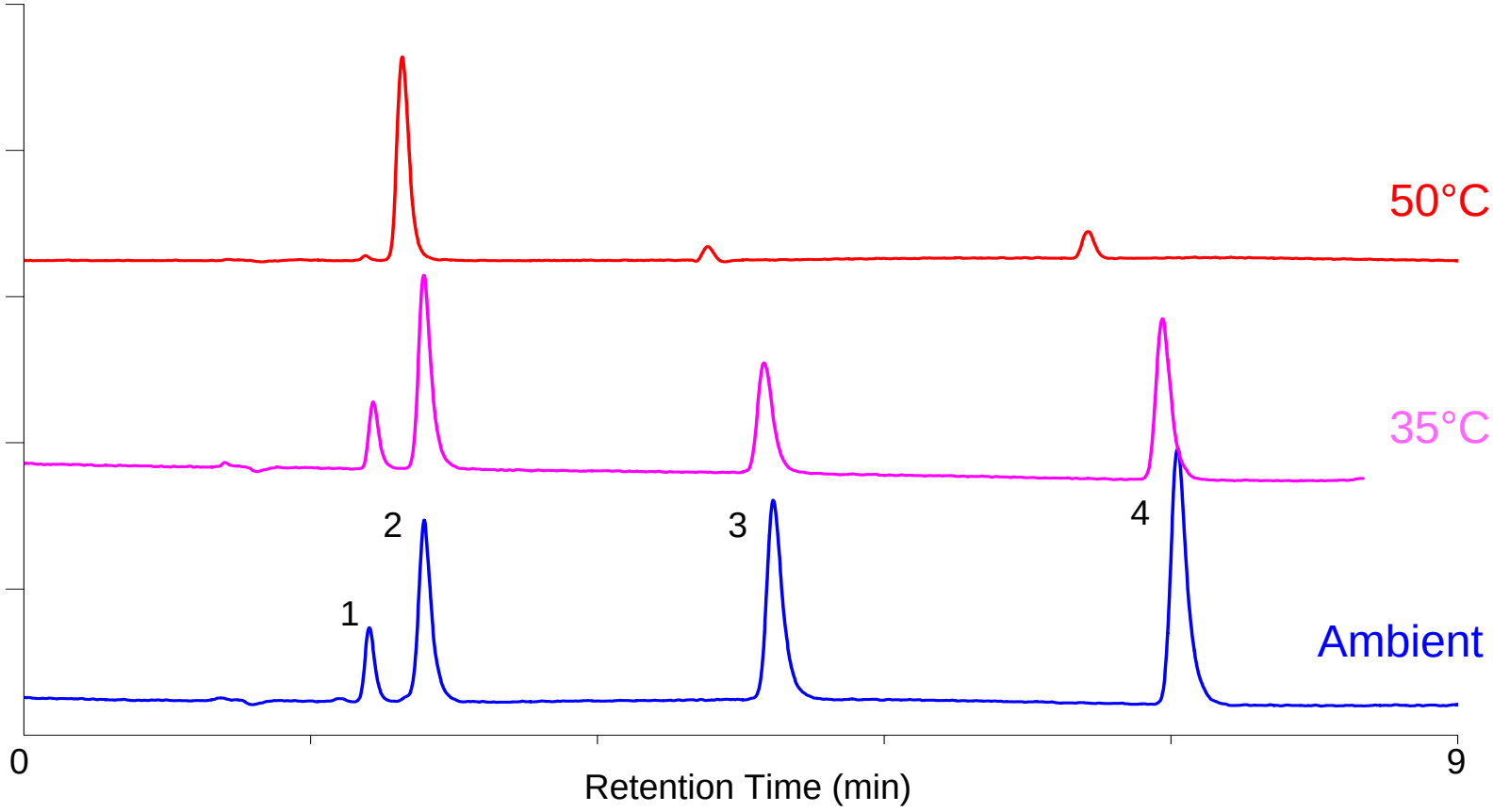

1. Serine
2. Glutamic Acid
3. Arginine
4. Proline
5. Valine
6. Methionine
7. Isoleucine
8. Leucine
9. Phenylanine
10. Tryptophan

# Volatile Application:
# Effect of Changing Temperature

| | |
|---|---|
| Column: | Adsorbosil C18 5µm, 150x4.6mm |
| Eluent A: | Water + 0.1% TFA |
| Eluent B: | ACN + 0.1% TFA |
| Gradient: | 60-90% B in 5 mins |
| Flow Rate: | 1.0ml/min |
| Detector: | PL-ELS 2100 |
| | (neb/evap=same temperature, gas=1.8 SLM) |
| Samples: | 1. Acetanilide |
| | 2. Indapamide |
| | 3. Ibuprofen |
| | 4. Dibutylphthalate |
| Note: | Peak 2 is non-volatile |
| | Peaks 1, 3 & 4 are relatively volatile |

# Volatile Application:
# Effect of Changing Temperature

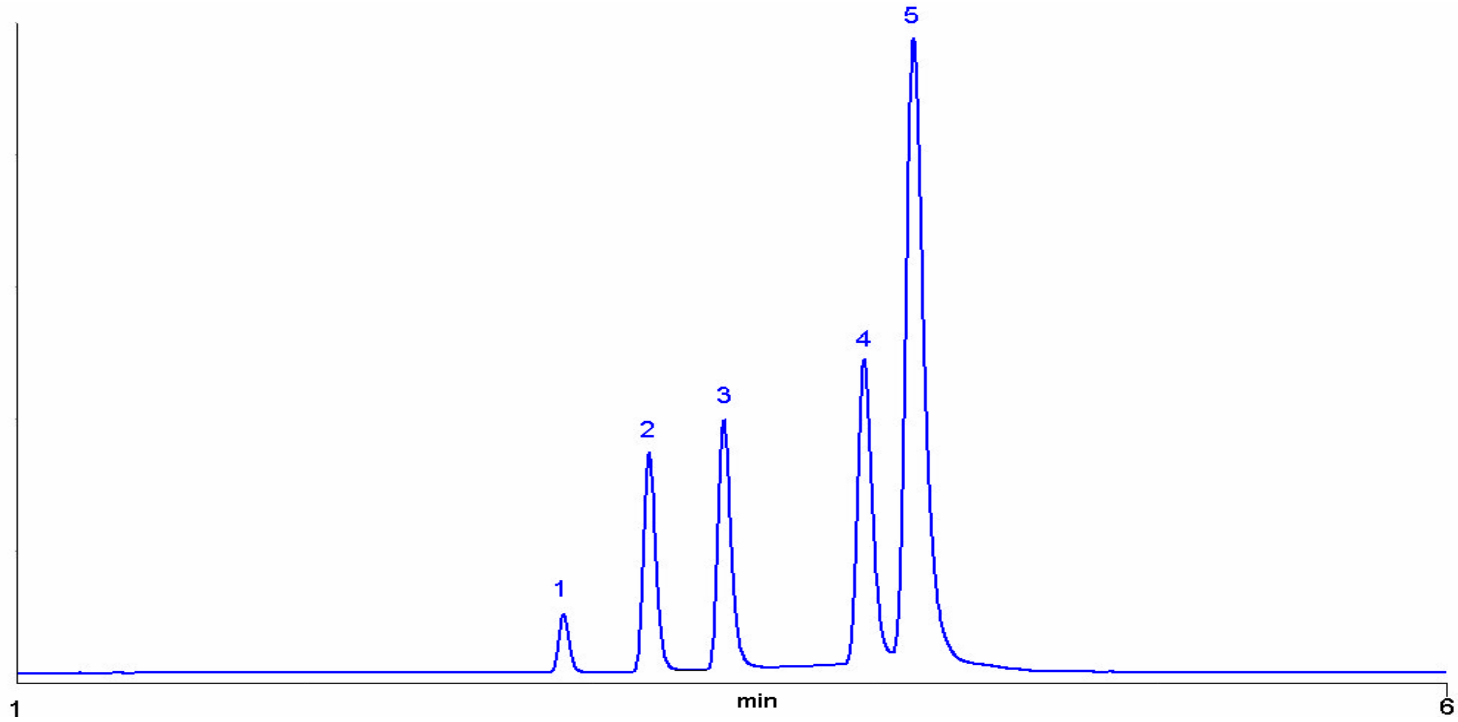
- At high temperature, peak 2 has better S/N but peaks 1,3 & 4 are not detected
- Running cold, all four peaks are detected



# Volatile Application: Phthalate Separation

| | |
|---|---|
| Column: | X-Terra C18 3.5µm, 30x2.1mm |
| Eluent A: | Water |
| Eluent B: | ACN |
| Gradient: | 0-100% B in 3 mins (hold 0.7 min) |
| Flow Rate: | 0.5ml/min |
| Detector: | PL-ELS 2100 |
| | (neb=25°C, evap=25°C, gas=1.6 SLM) |

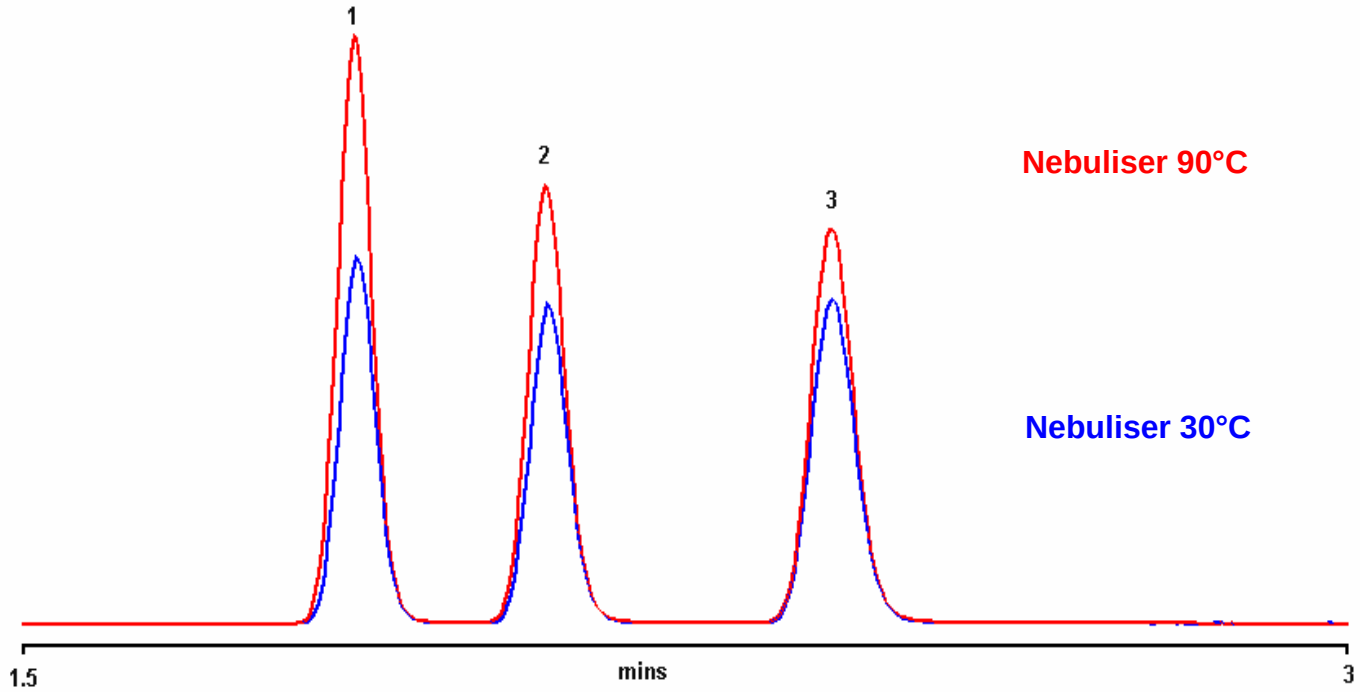
Samples
1. Diethylphthalate
2. Dipropylphthalate
3. Dibutylphthalate
4. Dipentylphthalate
5. Dioctylphthalate



# Volatile Application: Parabens Separation

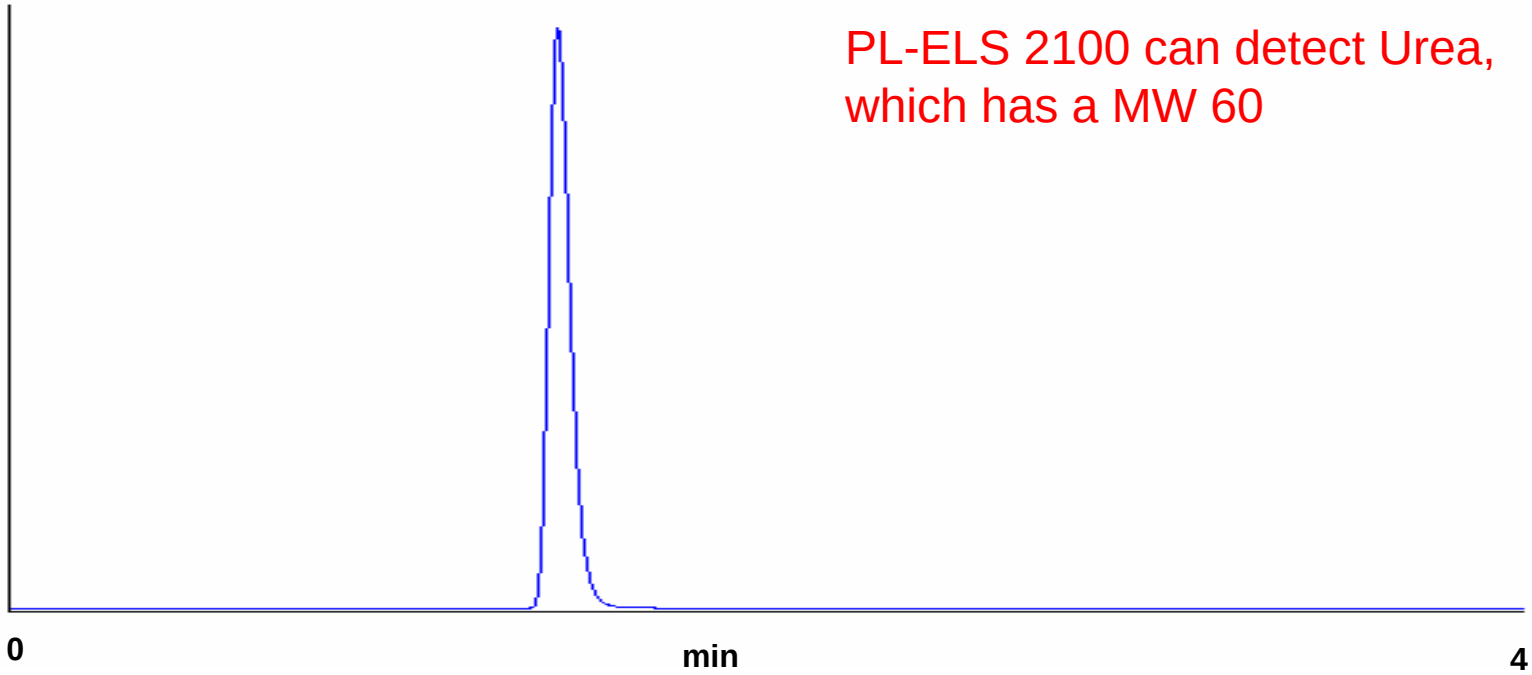
| | |
|---|---|
| Column: | Adsorbosil C18 5µm, 150x4.6mm |
| Eluent A: | Water |
| Eluent B: | ACN |
| Gradient: | 50-75% B in 7 mins |
| Flow Rate: | 1.0ml/min |
| Detector: | PL-ELS 2100 |
| | (Evap=30°C, Gas=1.0 SLM) |

Samples
1. Methylparaben
2. Ethylparaben
3. Propylparaben



# PL-ELS 2100
# Detection of Low Molecular Weight Compounds

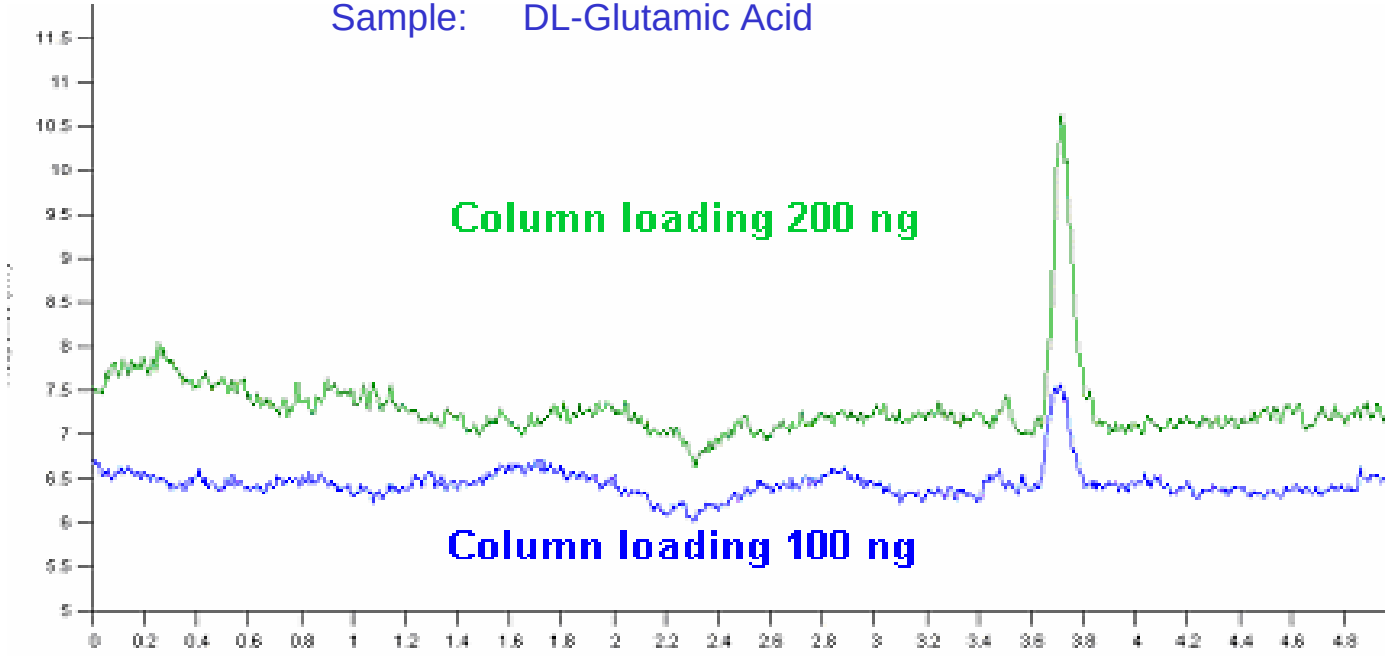
| | |
|---|---|
| Column: | Adsorbosil C18 5µm, 150x4.6mm |
| Eluent: | 60/40 Water/Acetonitrile |
| Flow Rate: | 1.0ml/min |
| Inj Vol: | 10µl |
| Detector: | PL-ELS 2100 |
| | (neb=30°C, evap=30°C, gas=1.6 SLM) |
| Sample: | Urea 1mg/ml |




PL-ELS 2100 can detect Urea, which has a MW 60

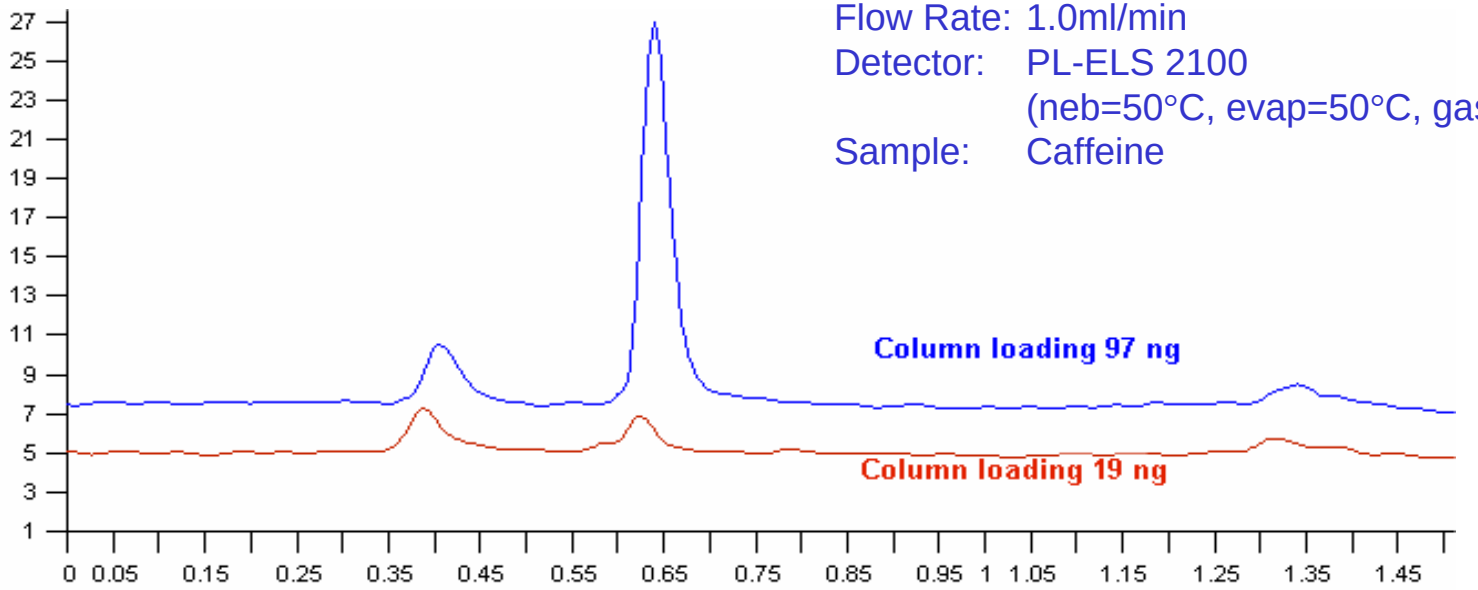
# PL-ELS 2100
# Sensitivity: Limits of Detection

| | |
|---|---|
| Column: | Spherisorb C18 5µm,150x4.6mm |
| Eluent A: | 99% Water + 0.1% TFA |
| Eluent B: | 1% ACN + 0.1% TFA |
| Flow Rate: | 0.5ml/min |
| Detector: | PL-ELS 2100 |
| | (neb=50°C, evap=70°C, gas=1.2 SLM) |
| Sample: | DL-Glutamic Acid |



# PL-ELS 2100
# Sensitivity: Limits of Detection

| | |
|---|---|
| Column: | ACE C8 3µm, 50x4.6mm |
| Eluent A: | 100% Water |
| Eluent B: | 100% ACN |
| Isocratic: | 50/50 A/B |
| Flow Rate: | 1.0ml/min |
| Detector: | PL-ELS 2100 |
| | (neb=50°C, evap=50°C, gas=1.4 SLM) |
| Sample: | Caffeine |



# Additional Benefits of the PL-ELS 2100

- Low temperature operation even for 100% water
- Extremely low dispersion for high resolution separations
- High eluent flow rates, up to 5ml/min
- Improved uniformity of response across a solvent gradient
- Rapid equilibration
- Extremely small footprint, stackable
- Ergonomic design
- Easy to use

# Les produits de nos partenaires
# Système LC ECD ALexys

ANTEC
LEYDEN

# Système LC ECD ALexys

ANTEC LEYDEN

Système intégré dédié à la détection électrochimique:

- Flexibilté : existe en version LC ou µLC
- Sensibilité:
- Ensemble du système optimisé pour la détection électrochimique
- Compatibilité CFR 21 Par 11

Composants du système :

- Pompe, amortisseur de pulsations
- Dégazeur
- Injecteur automatique analytique ou micro
- **DECADE II**
- Système de traitement de données

# Decade II
# Véritable plate forme de travail

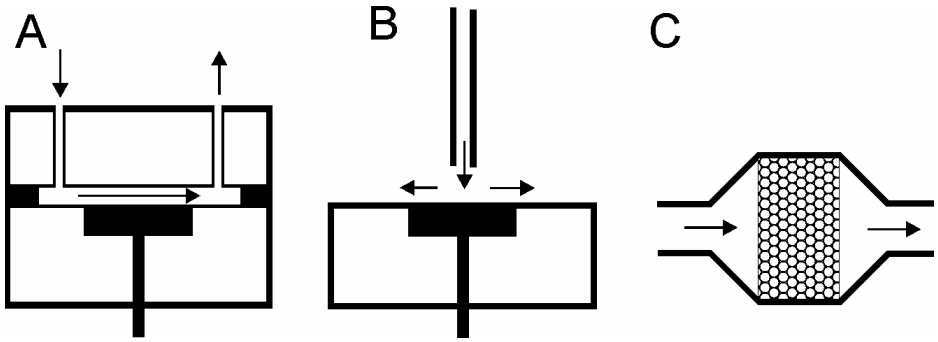
ANTEC LEYDEN

- Un four, Cage de faraday, abritant cellule de détection et colonne
- Pouvant contrôler une ou deux cellules de détection ( mode série ou parallèle)
- Concept de cellule original ( vaste choix d'électrodes de travail et de référence)
- Filtrage efficace du bruit de fond (ADF)

# Cellule ampérométrique VT-03

ANTEC LEYDEN

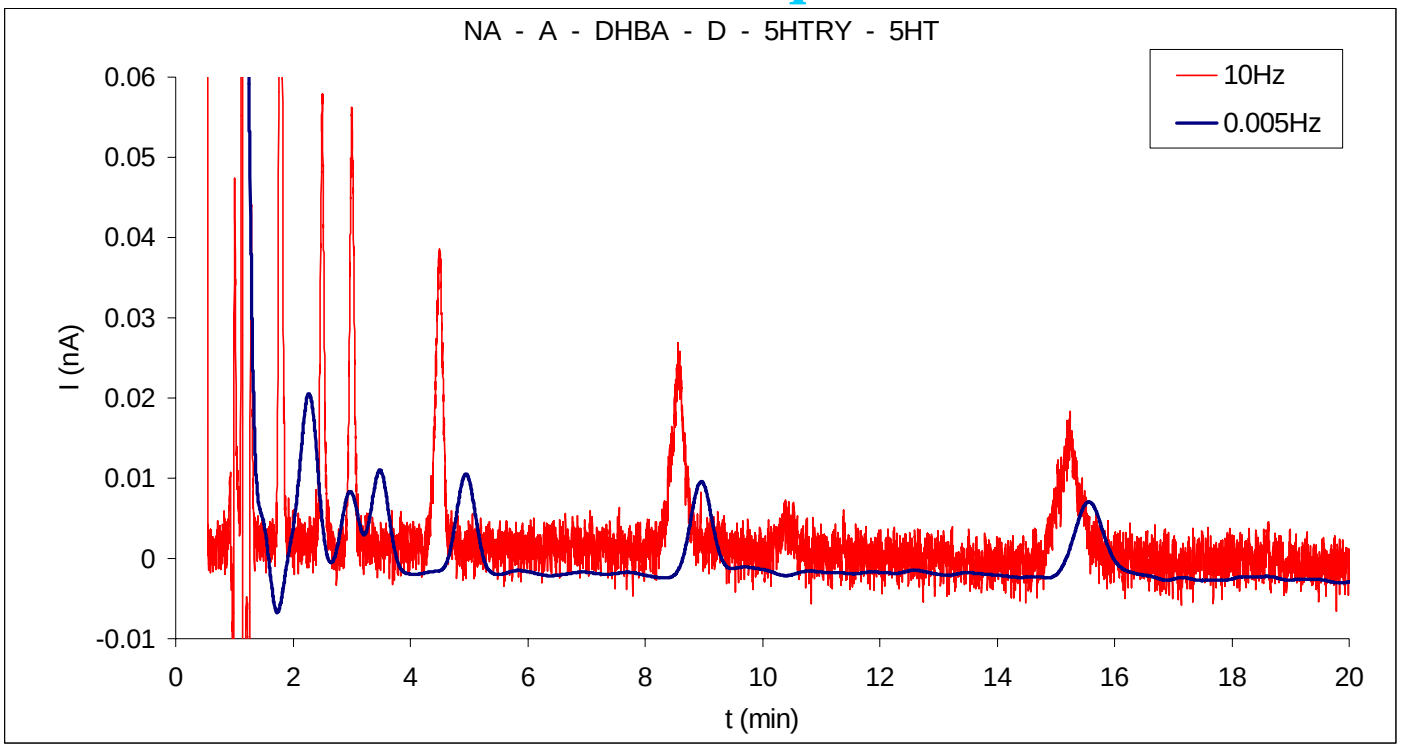
- Cellule de type Wall Jet
- Stabilisation rapide,
- Faible volume(0,3µL à 10nL)
- Sensibilité




A: couche mince, B wall jet, C Poreuse

# ADF avdanced digital filtration

## Filtre conçu spécialement pour la détection électrochimique




**Les basses fréquences ( pics) passent**

**Les fréquences élevées (bruit) sont bloquées**

# Applications types

- Analyse de traces nécessitant: séléctivité et sensibilité

- Applications en Micro dialyse ( Off line ou On line)

## Micro dialysate (frontal cortex + nucleus accumbens) settings

- ALEXYS system
  - flow 1 ml/min; injection 25 ul (ul pick-up mode)
  - column 10 x 0.46 cm 3 um ODS-2; spiked with 10 fmol DA + 5HT/25ul (= 4 x $10^{-10}$ M)
  - ADF 0.01 Hz up to 10 min, thereafter 0.002 Hz