

---

## Masterclass 1: The Science behind HPLC- 1 day

---

### 0900-1100

---

Understanding isocratic elution

- Void volume peak/solvent front and
- Peak retention time- factors affecting peak retention time
- Why separation occurs
- Questions and Discussion session

Factors affecting resolution in HPLC

- Relative retention time
- Selectivity
- Efficiency
- Peak Asymmetry
- Questions and Discussion session

### 1115-1300

---

HPLC Theory

- Thermodynamic properties
- Kinetic properties
- Band broadening
- Efficiency
- Questions/Discussion

Applying HPLC Theory to optimising separation

- Effect of peak size ratio on peak overlap
- Relationship between relative retention time and resolution
- Changing selectivity and solvent type
- Optimising efficiency
- Importance of particle size
- The theory behind ultra pressure HPLC
- Solvophobic Theory
- Acid-Base equilibria
- Questions/Discussion

### 1400-1700

---

Gradient elution

- Understanding gradient elution
- Effect of changing the gradient composition range or run time
- Effect of gradient dwell volume
- Measuring the gradient dwell volume of a HPLC system
- Gradient versus isocratic elution
- High pressure gradient mixing
- Low pressure gradient mixing
- Gradient profiles
- Ghost peaks
- Buffer and sample solubility
- Using gradient elution with a data system and autosampler
- Questions/Discussion