

**Discuss with examples the factors that are important in optimising an assay using HPLC**

- 1. Equipment Choice – type of pump and injection system and detector system for the analyte. Type of chromatography – packing material, ion exchange, ID of column.**
- 2. Type of analysis – qualitative – say drugs of abuse, or quantitative – say TPMT, Vitamins, TDM**
- 3. Solvent choice – including ability to separate as well as compatibility with equipment and detectors**
- 4. Gradient or standard chromatography**
- 5. Temperature – including newer ideas of temperature gradient**
- 6. Sample preparation – including discussion of the different approaches and their applicability to routine analysis**
- 7. Chromatography factors – resolution of the method, speed of the method from injection to injection, flow rate, detector settings**
- 8. Applicability to routine analysis – autosampler, pre-analytical**
- 9. Data handling and links to LIMS.**
- 10. Overall plan of method development – use of solvent triangle,**