HPTLC

Aim: quantitate DFS from single formulation of voveran using HPTLC

Principle:

* Works on sample principle as TLC
* Mobile phase flows through capillary action
* Separation relies on affinity of compounds to both phases
* Compounds in mobile move over surface of stationary
* Based on principle of adsorption or partition or combination of both chromatography
* Stationary affinity high 🡪 slow movement
* Stationary affinity low 🡪 fast movement
* Individual components appear as spots at levels on the plates

Requirements:

* Apparatus
  + Standard volumetric flask (50ml)
  + Pipettes (1ml, 5ml)
  + Hamilton syringe (100 micro litre)
* Chemicals
  + Methanol (HPLC grade)
* Miscellaneous
  + Mortar and pestle
  + Voveran 50mg tablet
* Instruments
  + CAMAG HPTLC with visionCATS software
  + Automatic sample applicator

Theory

* Sample prep:
  + Requires high conc solution.
  + Plate solvents much be non-polar and volatile.
  + Polar solvents are used for reverse-phase chromatography
* Layer selection:
  + For HPTLC it is very fine particle silica gel pre-coat.
  + Coat is Highly adsorbent
  + Plate is similar to TLC plate
  + Plates are 20x20 cm
  + 5x7.5 cm is used
* Pre-washing:
  + Washed with methanol
  + Used to remove water vapours and volatile impurities
* Conditioning:
  + Washed plates placed in over at 120c for 15-20 mins
* Sample application:
  + Sample application spot must not exceed 1mm in diameter
  + One technique is self-loading capillary
    - Small volume is applied to plate surface using platinum-iridium tube fused to glass tubing
* Pre conditioning:
  + High polar mobile phase 🡪 saturation necessary
  + Low polar mobile phase 🡪 saturation not necessary
* Mobile phase of HPTLC:
  + Determined through trial and error
* Chromatographic Development:
  + Linear development method:
    - Plate is positions vertically in container with mobile phase
    - Mobile phase fed by capillary action
    - Both sides may produce chromatograms
* Detection of spot and scanning:
  + Instrument attached to computer
  + Development of spots viewed as peaks at wavelengths of selected UV regions
  + Height and area are recorded by instrument as a percentage

Conclusion:

* Main features are
  + Low cost
  + Faster speed
  + Satisfactory precision
  + Accuracy
* DFS was quantified

HPLC

Aim: Separate DFS from combination (Dicloran A)

Principle:

* Reverse phase is most popular mode of HPLC
* Stationary phase is non-polar
* Mobile phase is polar
* Stationary phase is liquid coated inert solid (silica)
* Basic principle is partitioning of stationary phase and mobile phase
* Mobile phase flows through the column
* Retention time is longer for non-polar molecules
* Polar molecules elute faster
* More water in mobile phase 🡪 higher retention time
* Organic solvent in mobile phase 🡪 lower retention time