Blood is no plasma:

failing long-term frozen stability results for cyclosporin A in diluted whole blood

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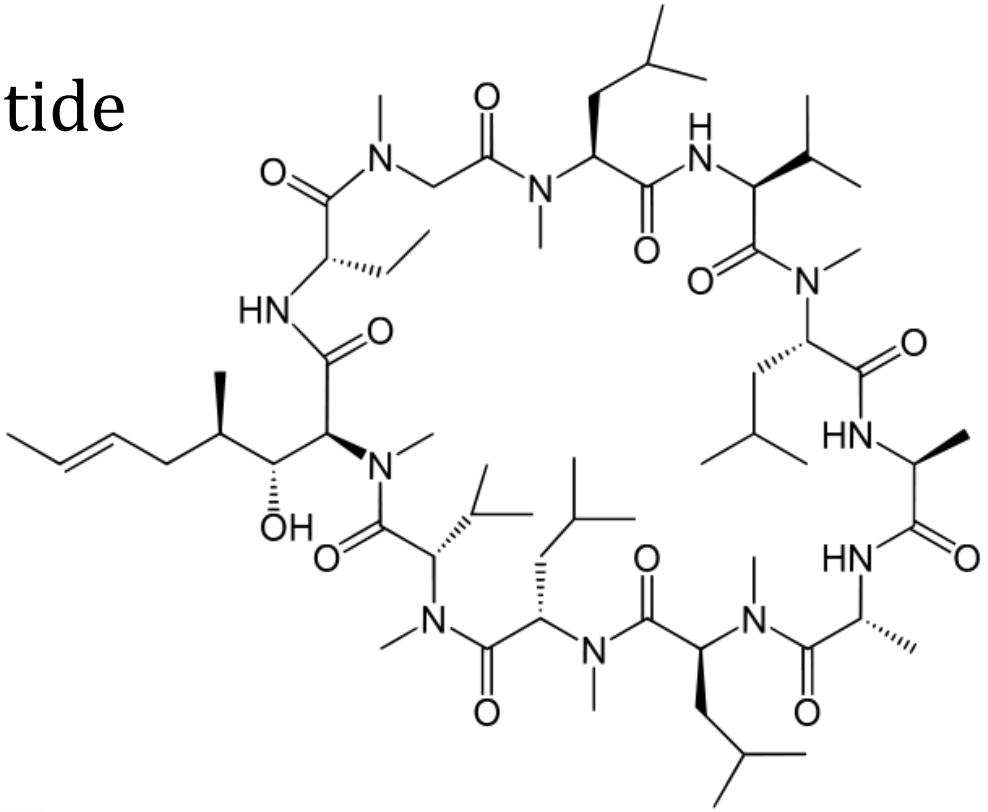
Background

Analyte:

- cyclosporin A
- cyclic undecapeptide
- MW: 1202.6
- logP: 3.6
- pKa: n.a.



Background

Analyte:

- cyclosporin A
- large temperature-dependent differences in partitioning between plasma and blood cells
- PK usually determined in blood (diluted with water to induce cell lysis)

Background

Method:

- explorative LC-MS/MS method (2003)
- concentration range 10-1000 ng/ml in EDTA whole blood, diluted with water (1:1, v/v)
- structural analogue IS: cyclosporin D
- extraction: 50 µL sample with 1 mL TBME
- LC: 50x2.1 mm C18 column at 65°C, acetonitrile/10 mM ammonium acetate (pH 6) (15:85, v/v)
- MS/MS: ESI+ m/z 1203.0 to m/z 675.6

Background

Qualification:

- 3-run accuracy and precision
- adequate bench-top (24 h), freeze/thaw (3 cycles) and autosampler (95 h) stability
- no long-term frozen stability assessed

Background

Method:

- updated in 2011
- concentration range 10-1000 ng/ml in EDTA whole blood, diluted with water (1:1, v/v)
- structural analogue IS: cyclosporin D
- extraction: 100 µL sample with 2 mL n-chlorobutane
- LC: 50x2.1 mm fluorophenyl column at 65°C, 3-min gradient of 40-95% acetonitrile in 0.1% formic acid
- MS/MS: ESI+ m/z 1203.0 to m/z 675.6

Background

Validation:

- 3-run accuracy and precision (bias and CV <7%)
- recovery 72-78% across the range
- adequate bench-top (23 h), freeze/thaw (3 cycles) and autosampler (97 h) stability
- acceptable frozen stability assessed at -20°C (13 days)

Background

Problem:

- second LTS assessment after 133 days at -20°C failed
- measured against freshly spiked calibration curve
- unacceptably high (>+20%) bias at high QC, but acceptable result at low QC
- consistent result after repetition

Your input

Our approach

Analysis:

- the effect apparently is concentration-dependent
- the effect apparently is time-dependent
- the bias is positive, so instability is unlikely
  - experimental errors were ruled out
  - accidental solvent evaporation was ruled out
  - increase in LLE recovery? If so: different for analyte and IS
- the matrix is unusual
  - could sample inhomogeneity play a role?

Our approach

Matrix:

- EDTA blood diluted 1:1 with water to induce cell lysis
- Differences in age of blank matrix at the time of stability assessment:
  - 13 days of storage: blank calibration matrix had also been stored frozen at -20°C for 13 days
  - 133 days of storage: blank calibration matrix had been stored frozen at -20°C for only 7 days

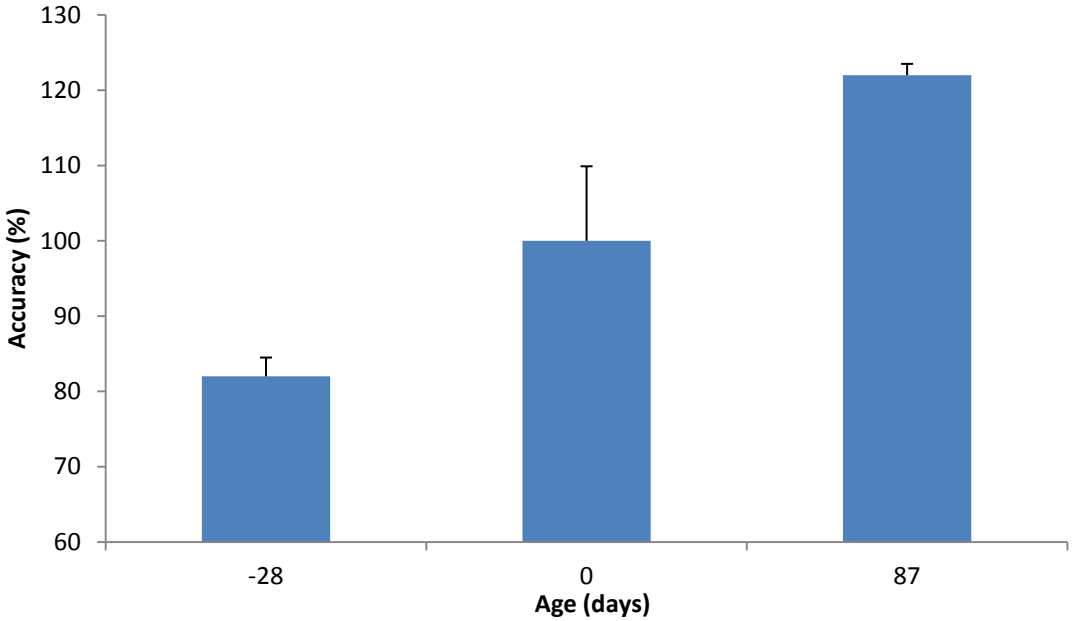
Our approach

Investigation:

- Test of accuracy as a function of age of blank calibration matrix
  - freshly spiked (high) QCs
  - analysed against one freshly spiked curve
  - using matrix with different relative ages
    - QCs in blank matrix 87 days older than blank matrix for cals
    - QCs in blank matrix of the same age as blank matrix for cals
    - QCs in blank matrix 28 days less old than blank matrix for cals

Our approach

Investigation:



Our approach

Investigation:

- LTS assessment after 185 days of storage at -20°C against fresh calibration curve spiked in 185-days old matrix
- excellent accuracy was found (bias <1%)

Our approach

Conclusions:

- the observed difference in accuracy is probably related to a change in extractability (recovery)
- cyclosporin A binds to sites on blood cells
- extractability may be enhanced over time, e.g. by increased lysis of blood cells during frozen storage
- the IS apparently does not correct for this effect
- a solution may be forced lysis during extraction (ultrasonication) to increase analyte recovery


PRA
A Clear Difference