Evaluation of stability

Experimental design of stability

- Time during which stability is tested, is important and can vary
  - Usually the autosampler stability injections are carried out overnight
  - Short time stability should be carried out at least over a stability analysis period of 48 h

Evaluation of stability

- At least at two concentration levels – low and high; matrix matched samples
- Bench-top stability/short-term stability – at room temperature or sample processing temperature
- Freeze-thaw stability – during three thawing cycles

- Stability should be evaluated at several different time points over the stability analysis period
- Samples should be analyzed in six replicates

Numerical expression of stability

- Evaluation via chromatographic peak areas

$$ST\% = \frac{S_t}{S_0} \cdot 100\%$$

- Evaluation via concentrations

$$ST\% = \frac{C_t}{C_0} \cdot 100\%$$

Numerical expression of stability

- The average percentage of analyte found in the sample under the specific conditions
  - The freshly prepared calibration standards are considered as containing 100% of the initial analyte content

Overcoming stability issues

Aspects to be considered while analytes are unstable

Measure	Challenge
pH control	Extract pH is crucial; Esterification of alcohols
Addition of stabilizers	Some additives itself are not stable Exact pipetting of stabilizers is required
Light protection	Working in dark or under yellow light
Reducing of sample processing time	Time critical process to be managed
Cold storage and handling	Every other step in the processing has to be cooled as well
Cold storage long-term (e.g. -70 ◦C; -80 ◦C)	Availability of -70 ◦C or -80 ◦C freezers
Derivatization (e.g. at sample collection)	Time critical and time consuming Completeness of reaction Matrix effects Reproducibility issues, IS or ILIS required