Quality by Design and Good Working Practices for CE

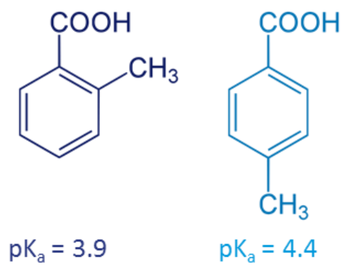
Cari Sänger – van de Griend,
Kantisto BV, Callenburglaan 22, 3742 MV Baarn, The Netherlands — cari.sanger@kantisto.nl
Uppsala University, Department of Medicinal Chemistry, Division of Analytical Pharmaceutical Chemistry, Biomedical Centre, Uppsala, Sweden
Australian Centre for Research on Separation Science (ACROSS), School of Chemistry, University of Tasmania, Hobart, Tasmania, Australia

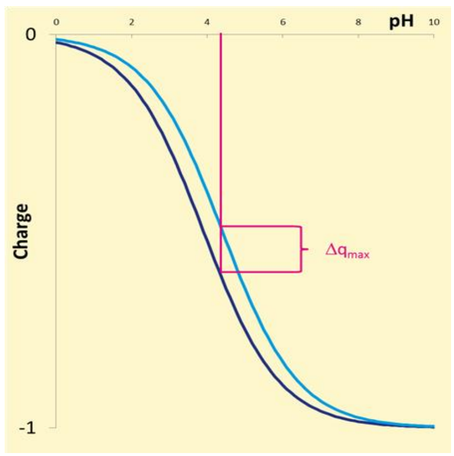
Introduction

Quality by Design means a scientific, risk-based, holistic and proactive approach. It has been successfully applied for some years within the pharmaceutical industry for formulation development and there is an increasing interest to apply QbD for analytical method development. ICH Q8 reads: “The degree of regulatory flexibility is predicated on the level of scientific knowledge provided.” and “... quality cannot be tested into products, i.e. quality should be built in by design.” This is just as valid for analytical methods.

“In theory, theory and practice are the same. In practice, they are not.” (Albert Einstein)

Quality should be build in by design



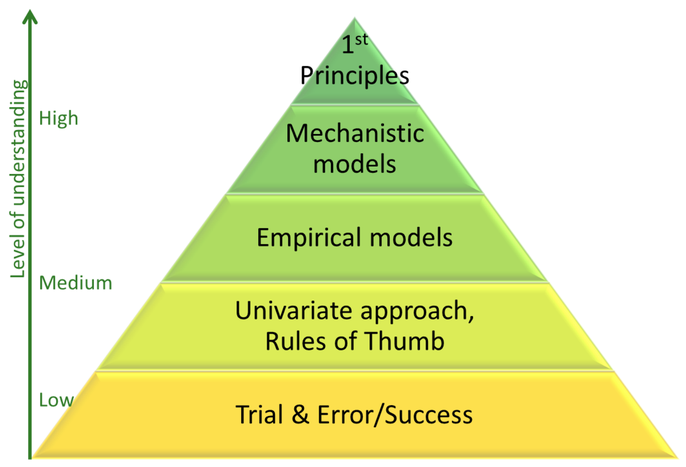


- Suppose two compounds such as positional isomers:
  - Same mass, same functional groups
  - Different pK_as
- Theory:
  - Best separation at largest charge/size ratio difference
  - Max charge/size ratio difference with pH between pK_as
- Practice:
  - Slight difference in pH, e.g. through buffer depletion, gives significant change in charge/size ratio difference
  - Relative mobility variability high
  - Not a robust situation!
  - Even worse if in highly-variable EOF region

BGE: heart of the method

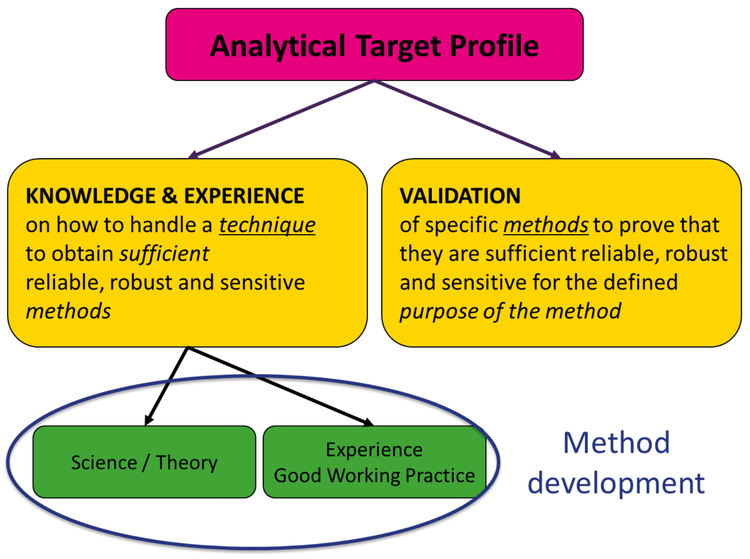
- The BGE is the core of the method stability and robustness
- pH:
  - Determines the charge on the analyte
    - Migration behaviour - separation
  - Determines electro-osmotic flow velocity
- Constant pH:
  - Check buffer capacity and amount of runs per vial pair
  - Buffer depletion: electrolysis of water at the electrodes
    Positive anode: $2\,H_2O \rightarrow O_2\,(g) + 4\,H^+ + 4\,e^-$
    Negative cathode: $2\,H_2O + 2\,e^- \rightarrow H_2\,(g) + 2\,OH^-$
  - Best buffering capacity at pK_a of the buffering component
  - Higher buffering capacity and better sample stacking at higher buffer concentrations, but also more current
- Precise recipes (see poster QbD and CE)
- CE quality water (18 MΩ, organics removed)
- Filter and/or degas

Analytical method understanding



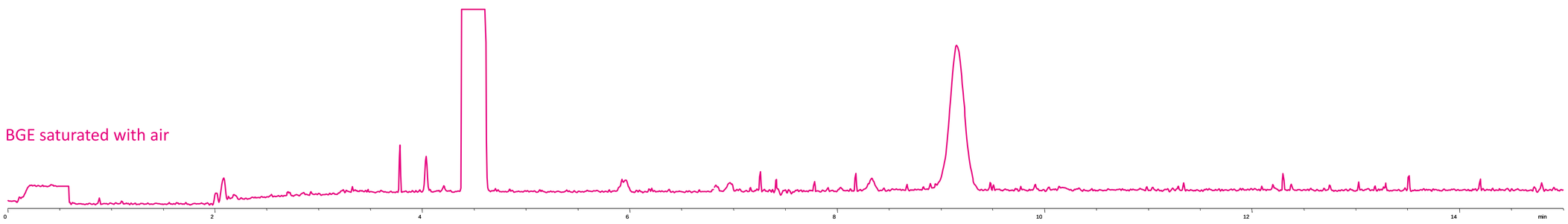
Analytical QbD

In order to design and develop robust and reliable methods from the start, one needs good knowledge about the fundamentals of the analytical technique used, and also on the good working practices of the technique.



Good Practice starts with cleaning!

- Do not remove cartridge without lowering lifts
- Keep all surfaces free of moisture and dried residue
- Clean the electrodes, pre-punchers, vial openers plus areas around at least once a week
  - Viscous BGEs (gel, CDs, urea): every sequence
  - Inspect and check for salt deposits
  - Electric toothbrush can be useful
- Wipe off capillary and electrodes before removing cassette

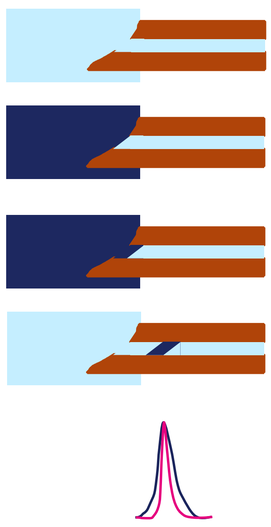


Quality by Design and Good Working Practices for CE

Kantisto
SEPARATION SCIENCES

"In theory, theory and practice are the same. In practice, they are not." (Albert Einstein)

Take care of your capillary



1. Capillary filled with BGE
2. Capillary placed in sample vial
3. Sample injection
4. BGE injection and start run

- Poorly cut or damaged end causes band broadening
- Condition of wall determines EOF
- Analyte - wall interaction causes peak tailing
- Keep detection window clean
- Remove polyimide of fused silica capillaries from capillary ends
  - Not for most coated capillaries (cIEF)!

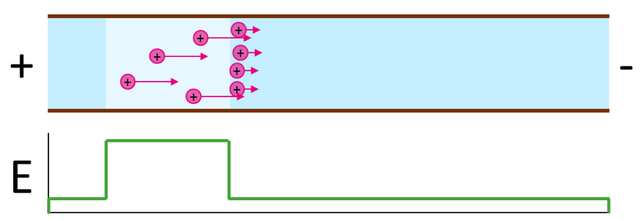
Modification of the capillary surface modifies the EOF

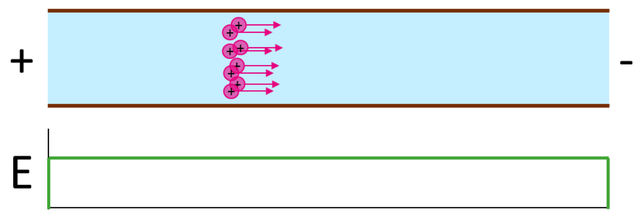
- Capillary history
  - Several common BGE components cannot be removed effectively from the capillary surface
  - One capillary, one application
  - After method development, test on fresh capillary
  - Take control over the EOF by using (adsorbed) coatings
- Proper conditioning procedure is part of method development
  - Watch out for too generic procedures - unnecessary long? - Not adequate?
  - Between injections: Rinse with BGE only, add on if needed
- The method SOP should describe capillary conditioning
- The method SOP should describe capillary storage
- Begin a sequence with two injections to stabilize the wall and EOF

Injection practice

- The composition of the standard should match the sample matrix
- Internal Standard to correct for injection volume variability
- The outlet vial should be a vial with a constant level, not the waste vial!
- Dip capillary in electrophoresis solution after sample injection to remove excess from outside
- Inject BGE plug after sample injection to prevent sample loss by thermal expansion when high voltage is switched on
- Reduce carry-over by burning polyimide off capillary ends
- Opportunities for stacking if sample conductivity is less than BGE conductivity:

Field amplified sample stacking





Voltage, current and temperature control

- Ramp the voltage at the start of the run, especially with very low-conducting samples and/or large injection volumes
- Record the current during the run. This is a powerful troubleshooting tool.
- Make an Ohm's plot to check if the Joule heating becomes excessive. The voltage/current point at which the heat becomes excessive depends on the cooling efficiency of the instrument, the actual BGE composition and the capillary dimensions.

Preconditioning step	Effects
BGE	• Cleans out sample components • Refreshes the BGE to avoid buffer depletion effects • Does not spoil your wall equilibrium
Applied voltage	• Stabilize the EOF • Opposite voltage to reduce carry-over
Water	• To bracket solvents that are not compatible
NaOH	• If harsher treatment of the capillary wall is needed (highly concentrated samples (analyte and/or matrix), sample components with strong wall interactions). • Not compatible with all capillary coatings
Strong acids, such as H_3PO_4 or HCl	• Harsher treatment than BGE or water, but without deprotonation of the silanolgroups of the wall. • Zeta-potential decreased (EOF slower) using the same pH in the BGE if acidic i.s.o. NaOH • Used for some coated capillaries
Organic solvents	• Other cleaning properties than aqueous solutions
Wait step	• Time to equilibrate
Dynamic coating solutions	• Do not always need to be added to the BGE, sometimes it suffices to flush in between runs
Dip capillary end	• Reduce carry-over