50 Ways to improve your method

Inspired by, and messing around with, Paul Simon's excellent text

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Introduction

The problem is all inside your head
She said to me
The answer is easy if you
Take it logically
I'd like to help you in your struggle
To be free
There must be fifty ways
To improve your method

She said it's really not my habit
To intrude
Furthermore, I hope my meaning
Won't be lost or misconstrued
But I'll repeat myself
At the risk of being crude
There must be fifty ways
To improve your method

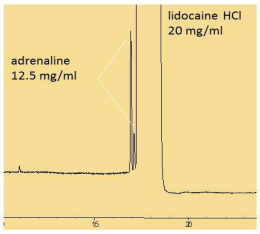
Experimental

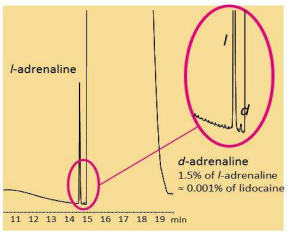
Capillary

1. Capillary history: One capillary, one application
2. After method development: Test ready method on a new capillary
3. Make sure you have straight capillary ends

4. Burn off the polyimide coating at the ends
5. Consider a dynamic coating of the capillary wall
   - To regulate the electroosmotic flow
   - To reduce sample interaction with capillary surface
   - Consider double or triple layer coatings
6. Optimise the capillary diameter for better sensitivity and sufficient Joule heat dissipation

- 75 µm capillary: 2^3 Full Factorial Design – best predicted result for lidocaine adrenaline solutions
- 50 µm capillary: reduced current, better resolutions: increase of CD concentration, injected volume and temperature possible

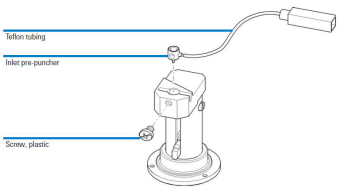




C.E. Sänger – van de Griend et al., J. Pharm. Biomed. Anal. 41 (2006) 77–83

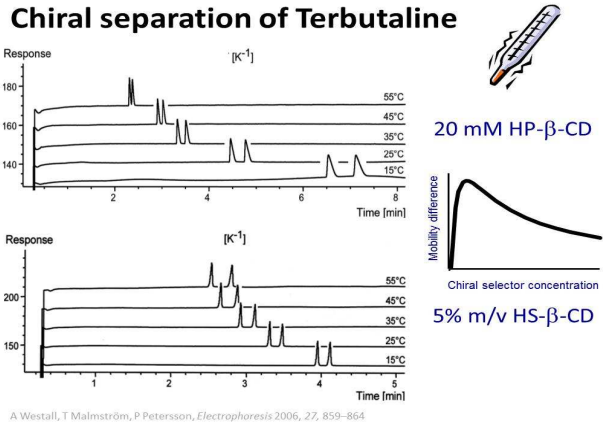
Maintenance

7. Clean the instrument regularly
8. Clean electrodes and prepunchers regularly



Temperature

9. Temperature differences between sample or buffer vials result in viscosity differences, resulting in migration time and injection precision variability. Let the vials stand in the autosampler for at least ½ h (e.g. during capillary conditioning and blank injection) before starting the main part of the sequence.
10. Temperature is a parameter that affects your separation, so optimise it:
    - Chiral separations especially
    - Effect on viscosity, pKa, equilibria etc.



A Westall, T Malmström, P Petersson, *Electrophoresis* 2006, *27*, 859–864

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Preconditioning

11. Optimize preconditioning in method development
    - Depends on your chemistry and samples
    - Examples of possible preconditioning steps:
        - BGE:
            - Cleans out sample components
            - Refreshes the BGE to avoid buffer depletion effects
            - Does not spoil your wall equilibrium
        - Applied voltage:
            - Stabilizes the EOF
            - Opposite voltage to reduce carry-over
        - Water to bracket solvents that are not compatible
        - Sodium hydroxide, 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.
        - Organic solvents
        - Wait-step
        - Dynamic coating solution(s):
            - Do not always need to be added to the BGE, sometimes it suffices to flush in between runs.
        - Detergent:
            - E.g. SDS for complex sample matrices, such as in biological samples
        - Dipping capillary end

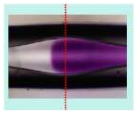
Injection

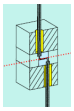
12. Match the composition of the standard with the composition of the sample
13. Use stacking as often as possible: dilute your sample with diluted electrophoresis solution
14. Use an Internal Standard
15. Avoid air bubbles at the bottom when using microvials
16. Use an outlet vial during injection with a constant level. This is NOT your waste vial!
17. Dip the capillary in electrophoresis solution or water after sample injection to remove excess from the outside
18. Inject a buffer/water plug after sample injection to prevent sample loss by thermal expansion when high voltage is switched on
19. Do spend time on developing the injection procedure, there is a lot to gain on sensitivity and reproducibility
20. Inject an intrinsic ITP stacking solution before/after the sample plug, e.g. a slower migrating, preferably non-absorbing compound with good solubility
21. Use stacking by sample dilution or the injection of a low-conductivity (water/diluted BGE) plug before/after the sample plug

Voltage and current

22. Ramp the voltage at start of run to prevent sample loss
23. When calculating mobilities, correct for ramping
24. Record the current during the run. This is a powerful troubleshooting tool
25. Make the current trace part of the method SOP

Detection





26. Optimise your detector settings, such as wavelength, rise time (peak width) and band width. Down to 190 nm is possible
27. Check both with and without reference wavelength
28. Use corrected peak areas, i.e. Peak area / migration time
29. Minimise integration errors by increasing sample concentration if possible
30. Use manual integration if needed. The integration software is not properly adapted for small CE peaks, which results in an overestimation of peak area
31. Use a larger capillary diameter for better sensitivity, if the current permits
32. Use a bubble cell or z-cell/high sensitivity cell for better sensitivity

Capillary dip after injection

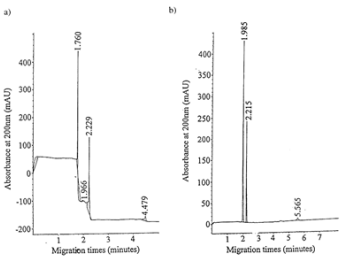
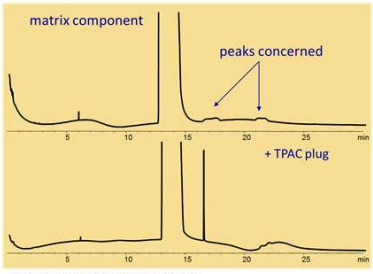


Figure 2.6 Analysis of a 3 component sample solution by MECC (without [Figure 2.6a] and with [Figure 2.6b] an additional capillary dip step in the method). Separation conditions: 20 mM LiDS/12.5 mM Na_2HPO_4/10 % v/v IPA, detection at 200 nm, 34 cm × 50 µm, 8 kV.

K.D. Altria, Analysis of Pharmaceuticals by Capillary Electrophoresis

Injection of TPAC plug after sample plug



TPAC = tetrapentyl ammonium chloride

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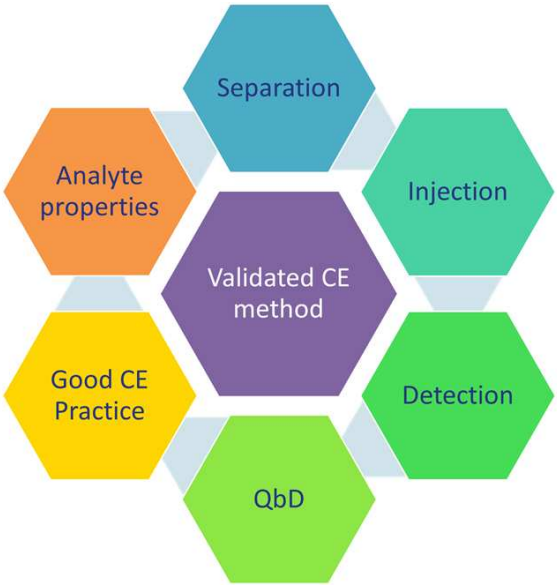
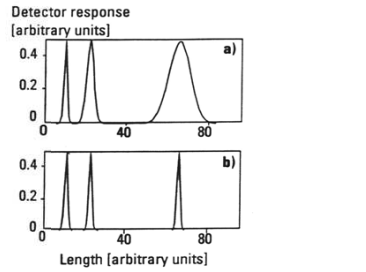




Detector response as (a) a function of time and (b) as corrected for differences in zone velocity.
Corrected peak area = peak area / migration time

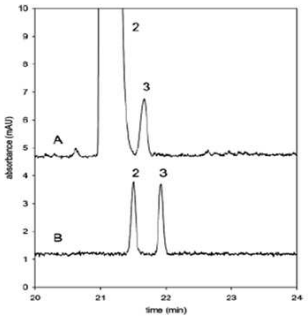
K.D. Altria, Chromatographia 35 (1993) 177-182

Samples and Standards

33. Use an internal standard for better reproducibility
34. Have the same vial fill levels for all samples
35. Dilution of samples sometimes gives increased sensitivity (due to conductivity effects)
36. The composition of the standards should match the samples. Viscosity differences give injection volume differences.
37. Use possible electrodispersion/stacking/transient-ITP effects of the sample matrix components to the full
38. The API should be present in validation (and method development) samples at nominal concentration, since the presence/absence of API affects quantification and identification of enantiomer (peak shape, resolution, migration times)

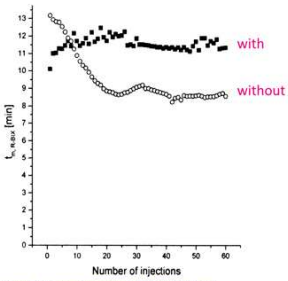
BGE solutions

39. Use precise recipes, E.g. 100 mM H_3PO_4 + 90 mM triethanolamine, results in pH 3.0
40. Reduce buffer depletion by swapping vials
41. Do not overfill the vials
42. Have the same liquid level for the inlet and the outlet vial
43. Sample loadability depends on BGE composition. High concentration gives better stacking (but also higher current)
44. Distinguish between a buffering co-ion and/or counter-ion
45. Use mobility matching to reduce EMD
46. Simple is beautiful. That is, more than 2 ions in the BGE result in ghost peaks
47. CE is very suitable for multi-factorial development, optimization and validation
48. Avoid the pH 4 – 7 range since the EOF will vary significantly with slight variation of the pH
49. If you do want to use the pH 4 – 7 range, use a coating
50. Do not chose a BGE pH close to the pK_a of your compound



P.G. Muijselaar et al., Electrophoresis 25 (2004) 2854-2859

Reduce buffer depletion by swapping vials



T Sokoliešj, G Köhler, Electrophoresis 26 (2005) 2330-2341

Concluding Remarks & Acknowledgement

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Over the years, we all, as participants of the CE user network, have contributed with our knowledge and experience to the Good Working Practices for the Capillary Electrophoresis techniques. This collection of knowledge and experience is paramount to the implementation of the CE techniques in industry, combined in the training of all technicians, scientists, managers and regulatory CMC people.

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