

High throughput screening of pKa by capillary electrophoresis and mass spectrometry (CE/MS) and long-term validation

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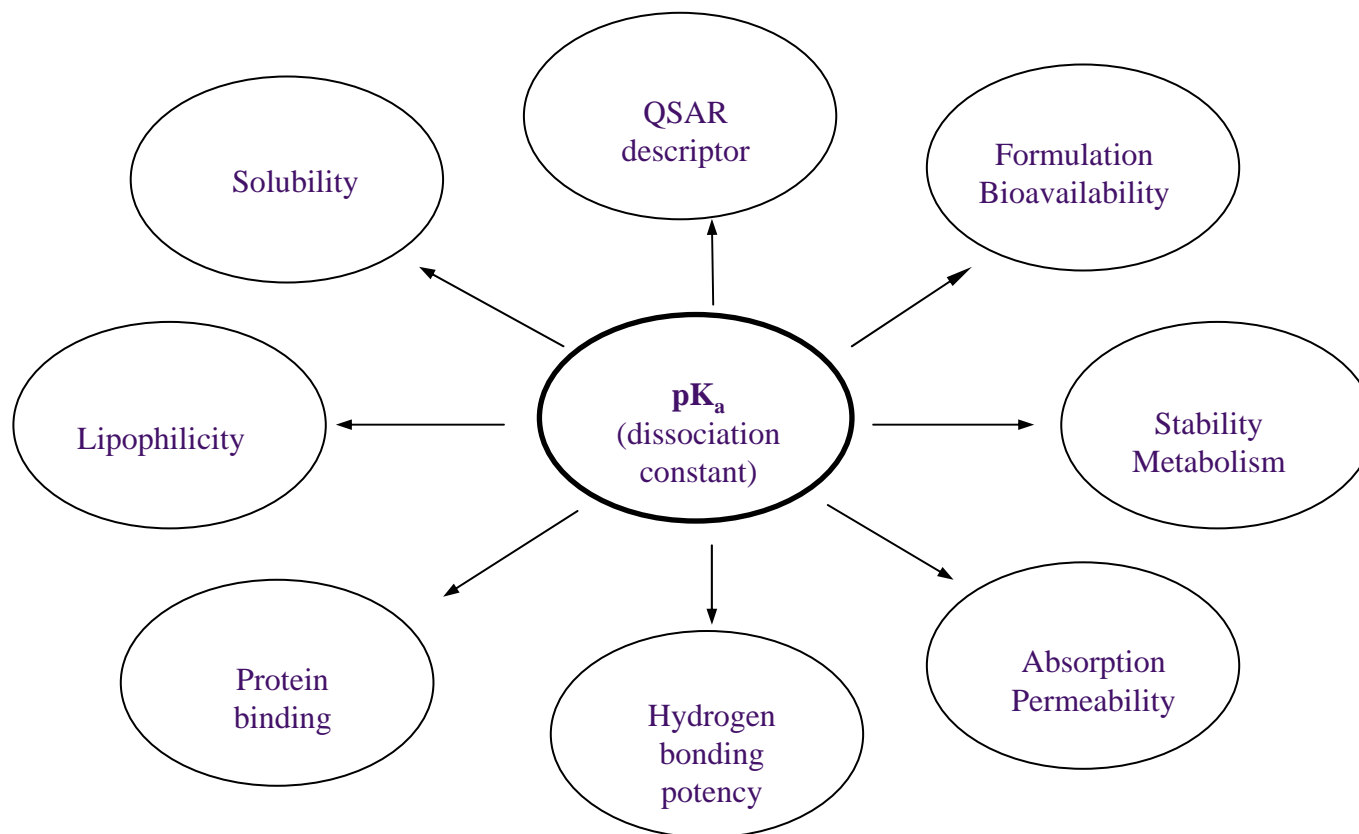
5th Physchem Forum, Stevenage, UK, June 19th

Outline

- Why screening pKa
- Advantages of CE/MS over established methods
- High throughput pKa screening method by CE/MS
 - Effects of buffer type and ionic strength on pKa
 - Comparison of CE/UV and CE/MS
 - Effects of pressure on migration times/sensitivity/mobility
- Comparisons of measured pKa and ref. and predicted values
- Reproducibility and accuracy and long-term validation
- Summary

Screening for pKa, why?

- Info about change in charge of molecule.....



Is CE/MS better technique for pKa than others?

- Sirius GLpKa – well established and widely used method !
- pION's Gemini for routine pKa (\$500/cdp, \$760/cpd/co-solvent)
- CE/UV (CombiSep), commercially available
- CE/MS (new technology), AZ, Mölndal, 2003 published.

H. Wan, A. Holmén, M. Någård, W. Lindberg, J. Chromatogr. A. 979 (2002) 369.

H. Wan, A. Holmén, Y. Wang, W. Lindberg, M. Englund, M. Någård, R. Thompson, Rapid Commun. Mass Spectrom. 17 (2003) 2639.

Advantages of CE/MS over established methods

Specification	CE/MS	Titration/UV (D-PAS)
Amount of cpd	1 μg , or 1 μL of 10 mM solution	1-2 mg (titration) 3 μL of 10 mM DMSO stock (Sirius 3T)
Concentration	1-10 μM (100 μL)	> 20 μM (UV)
Purity required	No	Yes
Co-solvent	No	Yes, for poorly soluble cpds
Accuracy	< \pm 0.2	\pm 0.02-0.2?
Throughput capacity	>150 cpds/seq.(6h)	4 min/cpd (Sirius T3)
Limitation	Poor ionization (ESI)	Impurity? poor solubility (precipitation), Ionization group close to chromophore

Principle of pKa determination by CE

pKa \leftrightarrow 50% ionization

$$K_a^{\text{th}} = \frac{\{H^+\}\{A^-\}}{\{HA\}}$$

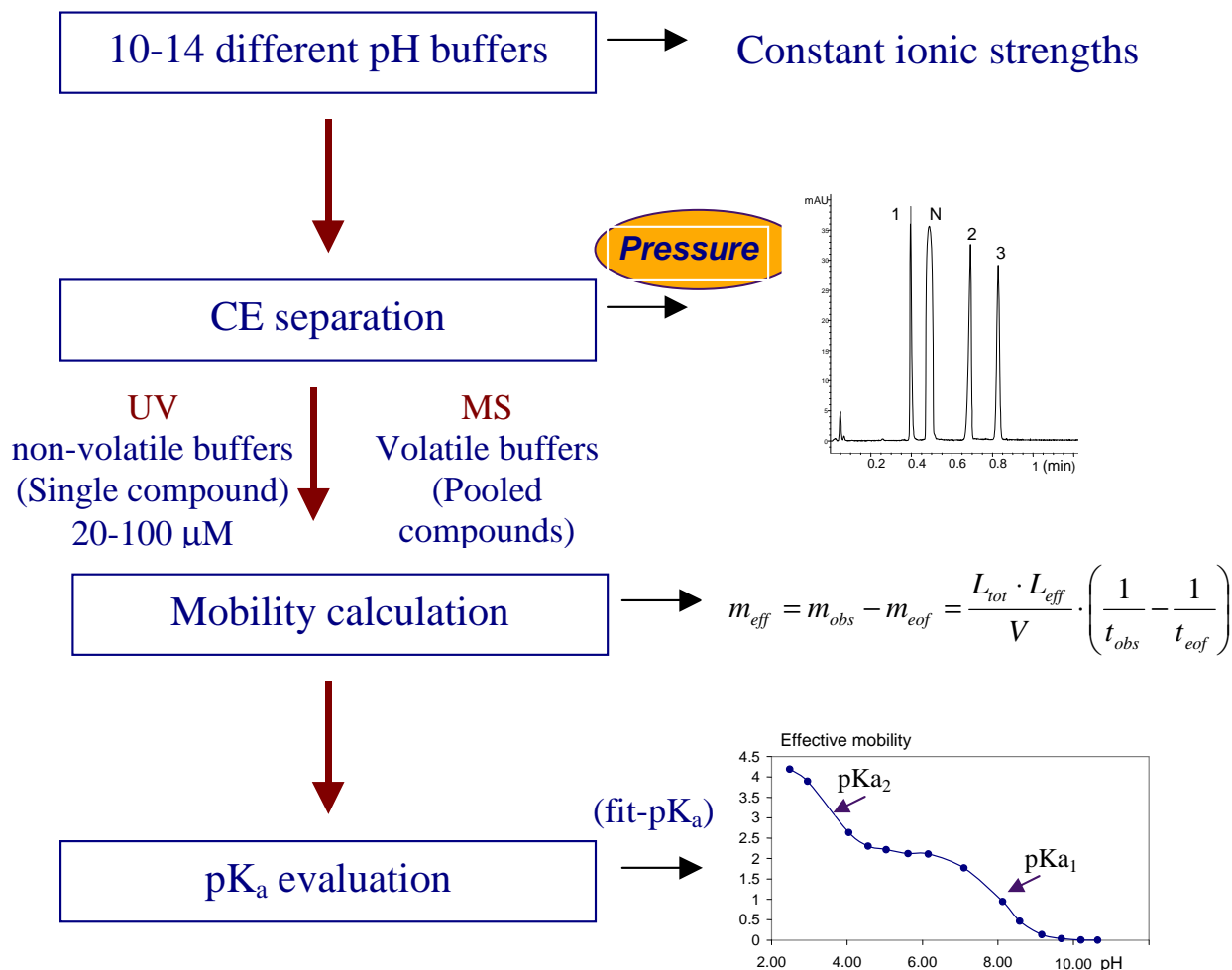
$$pK_a^{\text{th}} = \text{pH} - \log \left[\frac{m_{\text{eff}}}{m_m - m_{\text{eff}}} \right] + \frac{0.5085z^2\sqrt{I}}{1 + 0.328\alpha\sqrt{I}}$$

Equations used for pKa calculation

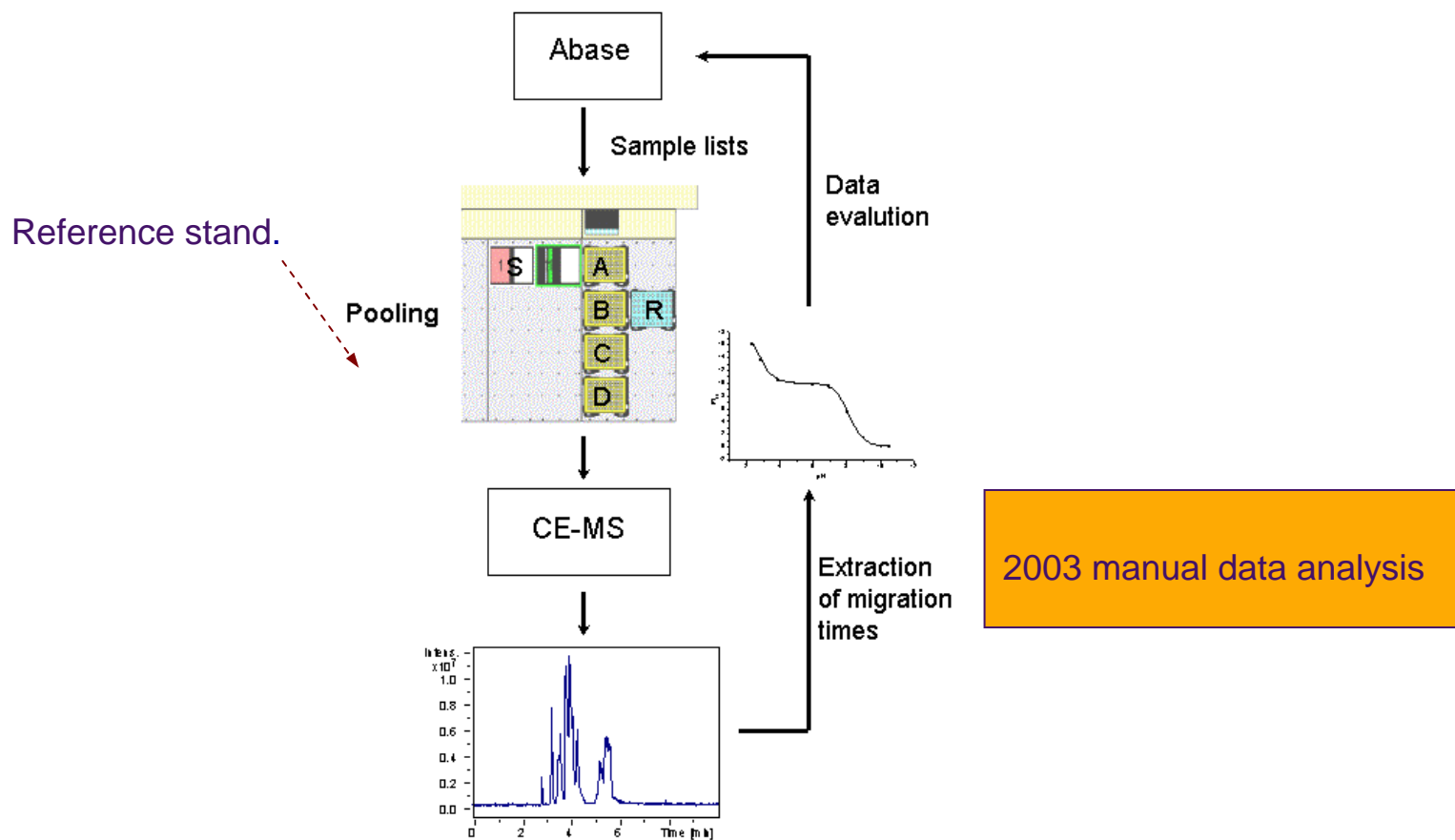
Ionizable type	Model equation
Monobase	$m_{\text{eff}} = \frac{M_b 10^{-\text{pH}}}{10^{-\text{pK}_a} + 10^{-\text{pH}}}$
Monoacid	$m_{\text{eff}} = \frac{M_a 10^{-\text{pK}_a}}{10^{-\text{pK}_a} + 10^{-\text{pH}}}$
Dibase	$m_{\text{eff}} = \frac{M_{b2} [10^{-\text{pH}}]^2 + M_{b1} 10^{-\text{pK}_{a1}} 10^{-\text{pH}}}{[10^{-\text{pH}}]^2 + 10^{-\text{pK}_{a1}} 10^{-\text{pH}} + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}}}$
Diacid	$m_{\text{eff}} = \frac{M_{a1} 10^{-\text{pK}_{a1}} 10^{-\text{pH}} + M_{a2} 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}}}{[10^{-\text{pH}}]^2 + 10^{-\text{pK}_{a1}} 10^{-\text{pH}} + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}}}$
Monoacidic monobasic ampholyte	$m_{\text{eff}} = \frac{M_{b1} [10^{-\text{pH}}]^2 + M_{a1} 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}}}{[10^{-\text{pH}}]^2 + 10^{-\text{pK}_{a1}} 10^{-\text{pH}} + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}}}$
Tribase	$m_{\text{eff}} = \frac{M_{b3} [10^{-\text{pH}}]^3 + M_{b2} 10^{-\text{pK}_{a1}} [10^{-\text{pH}}]^2 + M_{b1} 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pH}}}{[10^{-\text{pH}}]^3 + 10^{-\text{pK}_{a1}} [10^{-\text{pH}}]^2 + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pH}} + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pK}_{a3}}}$
Triacid	$m_{\text{eff}} = \frac{M_{a1} 10^{-\text{pK}_{a1}} [10^{-\text{pH}}]^2 + M_{a2} 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pH}} + M_{a3} 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pK}_{a3}}}{[10^{-\text{pH}}]^3 + 10^{-\text{pK}_{a1}} [10^{-\text{pH}}]^2 + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pH}} + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pK}_{a3}}}$
Diacidic monobasic ampholyte	$m_{\text{eff}} = \frac{M_{b1} [10^{-\text{pH}}]^3 + M_{a1} 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pH}} + M_{a2} 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pK}_{a3}}}{[10^{-\text{pH}}]^3 + 10^{-\text{pK}_{a1}} [10^{-\text{pH}}]^2 + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pH}} + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pK}_{a3}}}$
Monoacidic dibasic ampholyte	$m_{\text{eff}} = \frac{M_{b2} [10^{-\text{pH}}]^3 + M_{b1} 10^{-\text{pK}_{a1}} [10^{-\text{pH}}]^2 + M_{a1} 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pK}_{a3}}}{[10^{-\text{pH}}]^3 + 10^{-\text{pK}_{a1}} [10^{-\text{pH}}]^2 + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pH}} + 10^{-\text{pK}_{a1}} 10^{-\text{pK}_{a2}} 10^{-\text{pK}_{a3}}}$

Graphs and table from Miller, *Electrophoresis* 2002, 23, 2833

High-throughput pKa screening by CE/MS

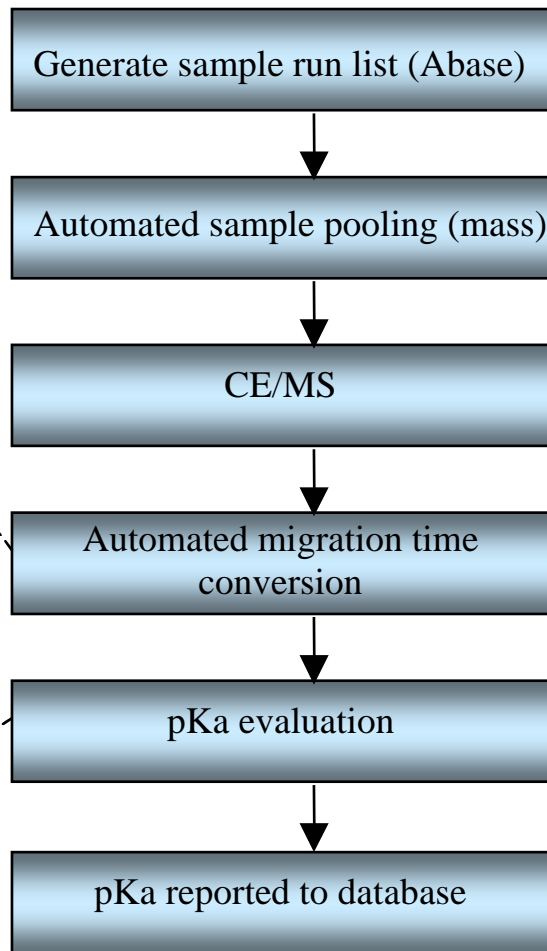
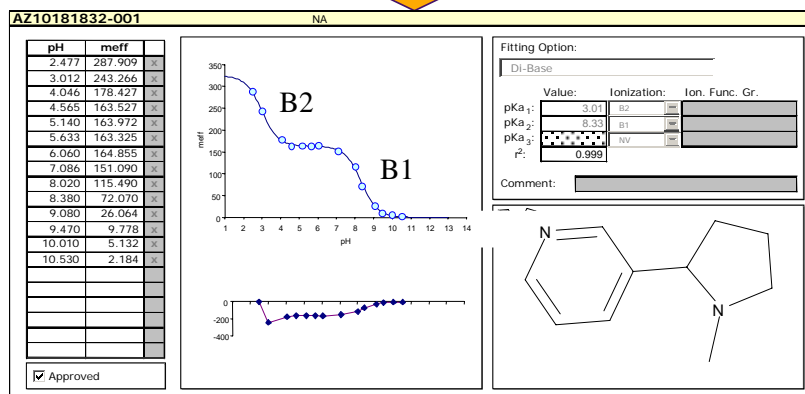
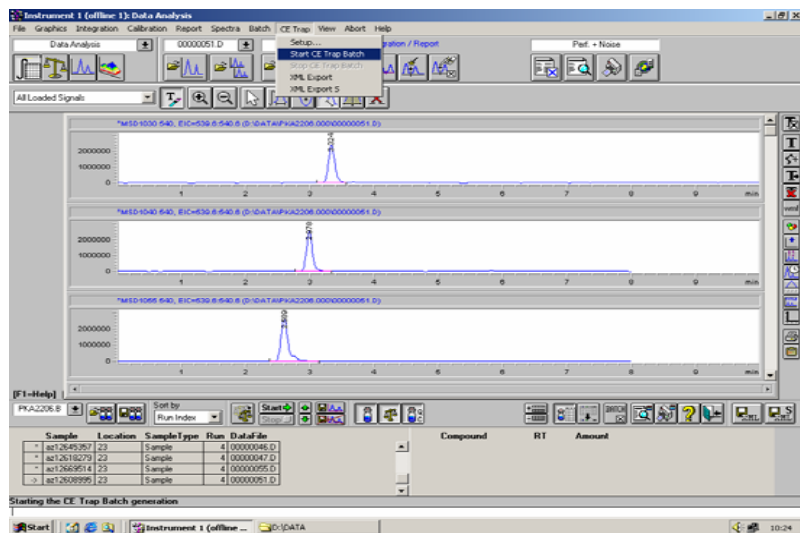


Sample pooling and data analysis flow scheme



H. Wan, A. Holmén, Y. Wang, W. Lindberg, M. Englund, M. Någård, R. Thompson,
Rapid Commun. Mass Spectrom. 17 (2003) 2639.

Current pKa screening - integrated & automated assay



CE/MS instrumentation

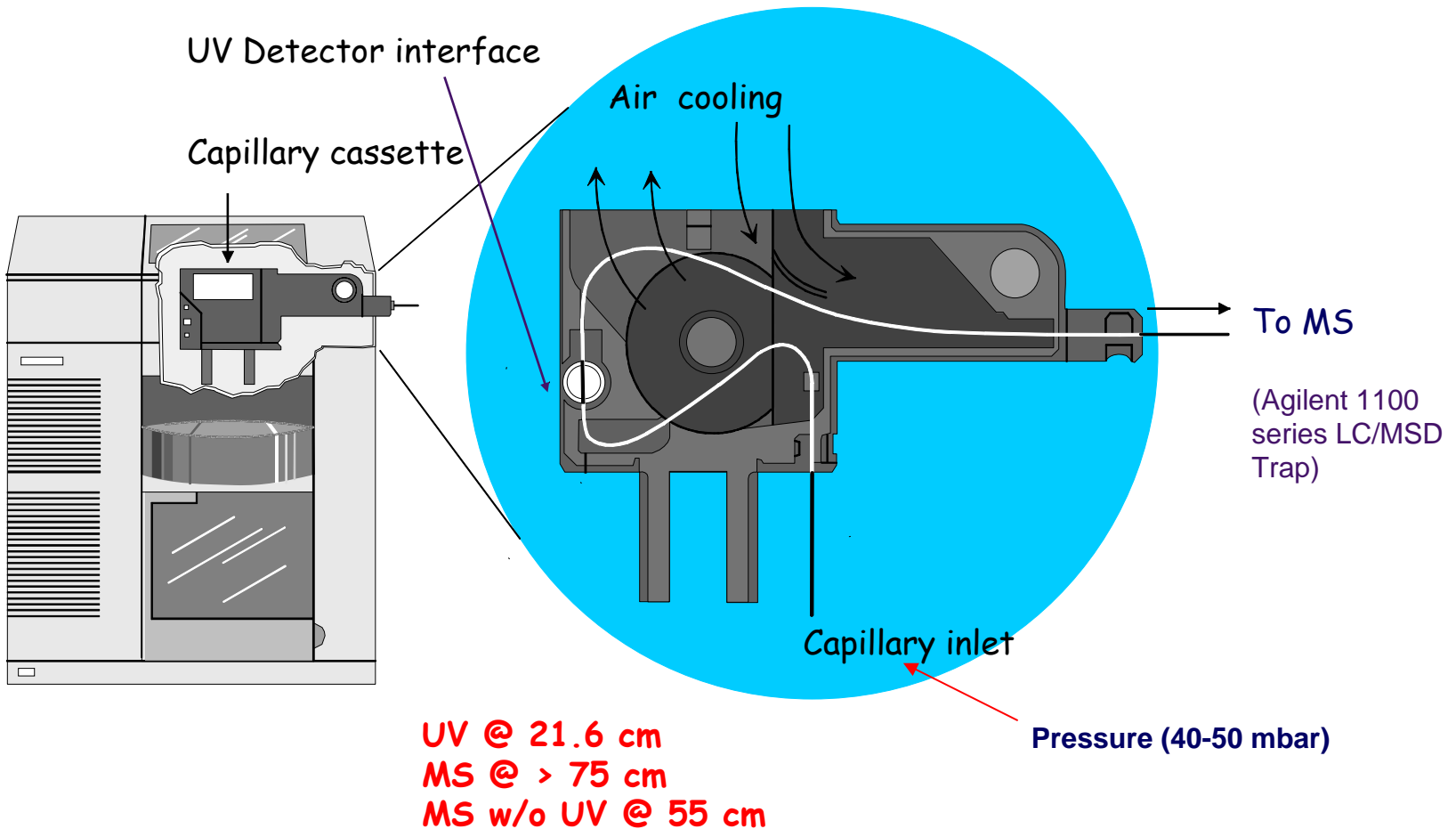


- Higher initial investment
- Long-term savings

HPCE^{3D} , 1100 series LC/MSD trap SL (Agilent)

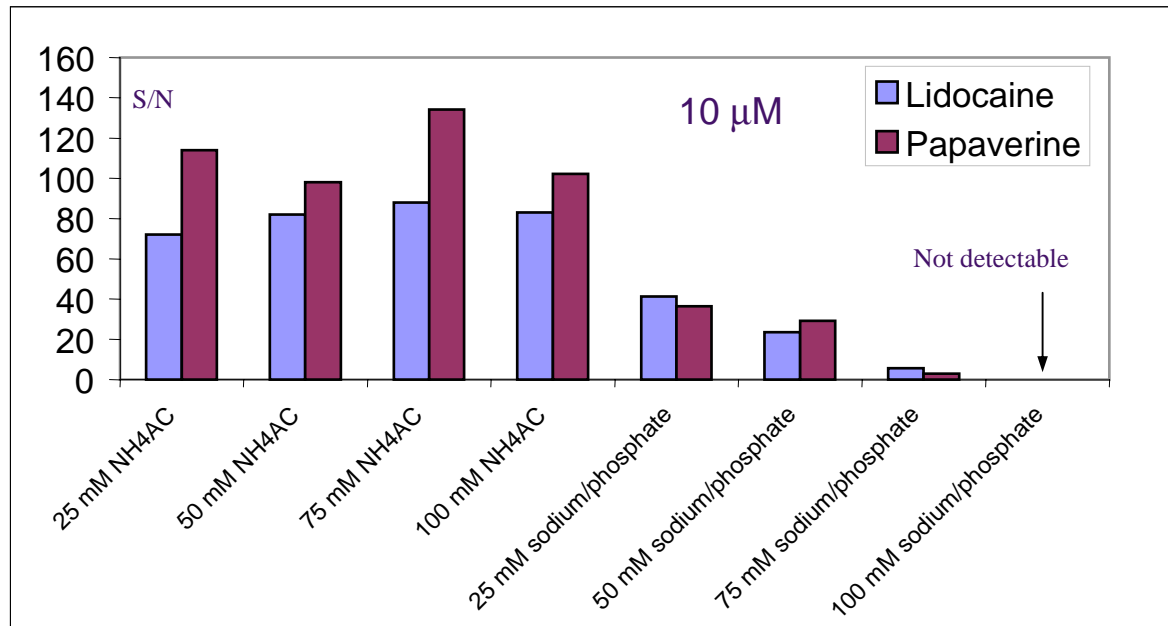
- Untreated fused silica capillary (50-60 cm/50 SEK) can be used as long as it works.
- Buffers without filtration (stock solutions can be used up to >3 years).
- Sheath liquid (1 L) can be used for more than 6 months (99% recycling).

On-line CE/UV/MS experimental setup



Courtesy from Agilent (with small modifications)

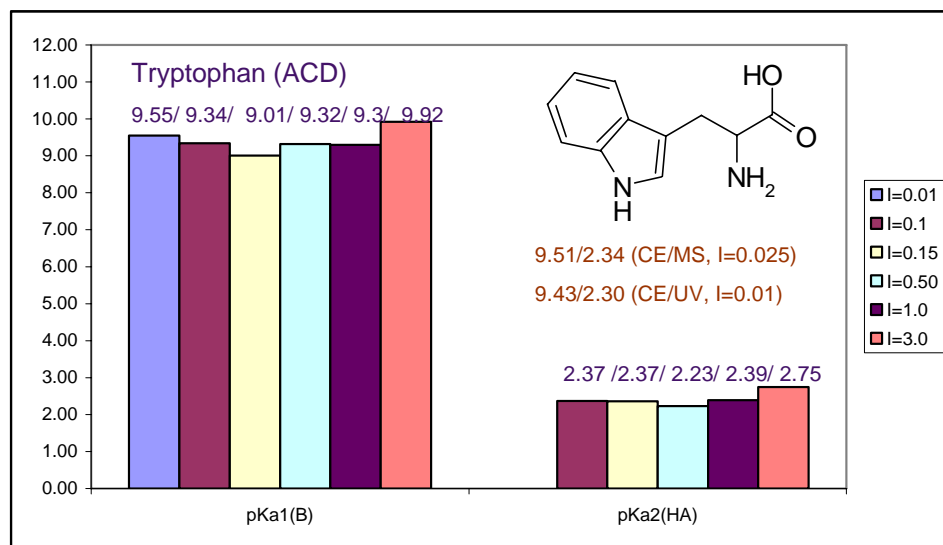
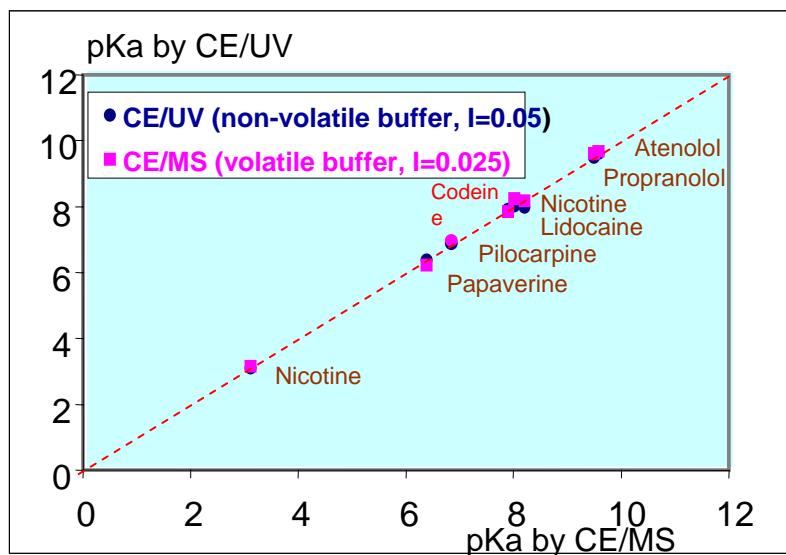
Sensitivity from volatile vs non-volatile buffers in CE/MS



- Non-volatile buffer decreased signals at higher concentration (ion suppression)
- Ion source contamination by non-volatile buffer

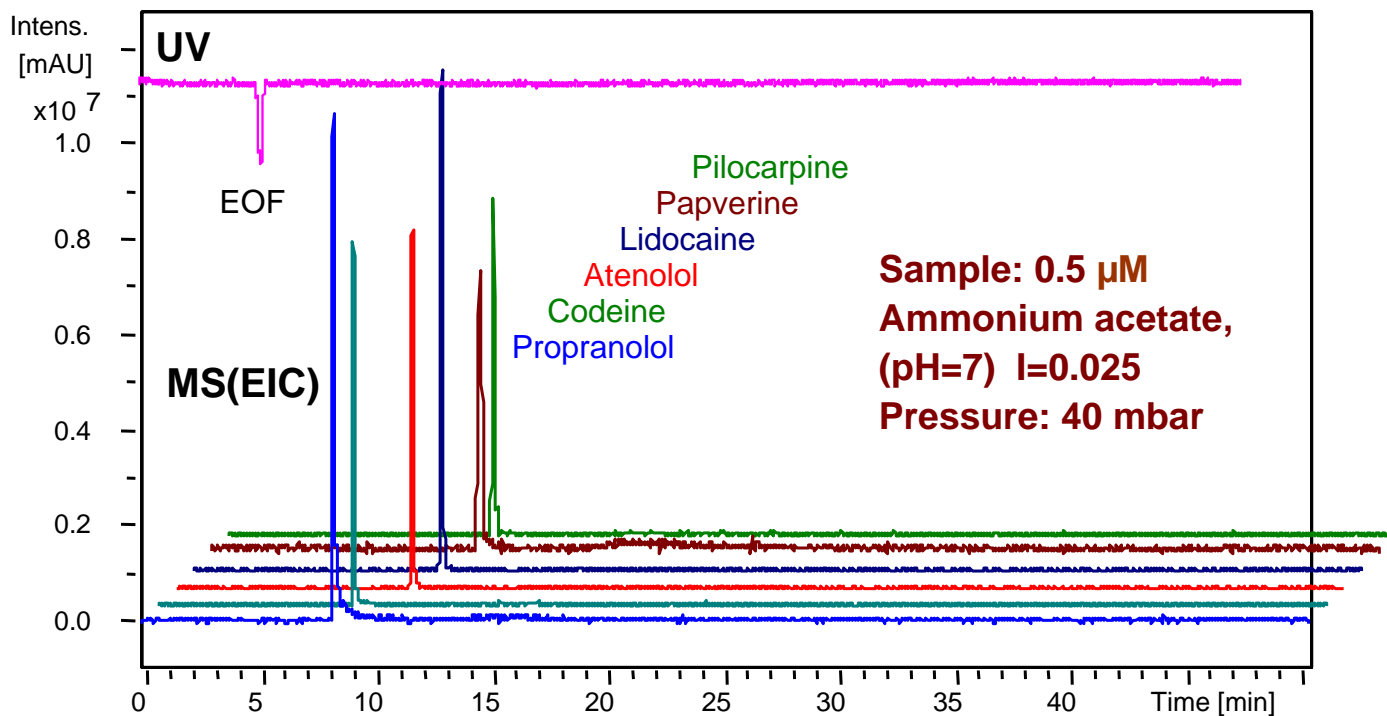
Effects of buffer type and ionic strength on pKa

$$pK_a^{th} = pH - \log \left[\frac{m_{eff}}{m_m - m_{eff}} \right] + \frac{0.5085z^2 \sqrt{I}}{1 + 0.328\alpha \sqrt{I}}$$



- Titration method uses physiological buffer with ionic strength at 0.15 M.
- Ionic strengths (0.025 - 0.15 M) have a small effect on pKa ($\Delta pK_a=0.064$)

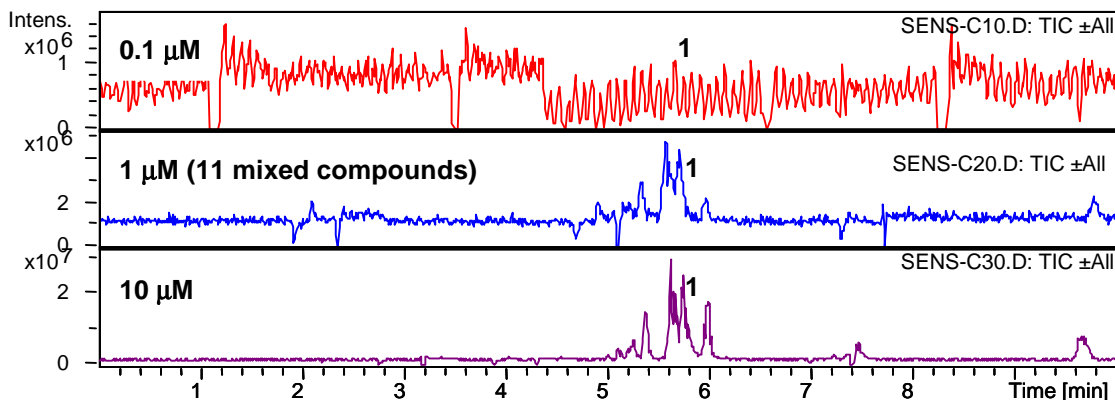
Comparison of UV and MS (Ion trap) sensitivity



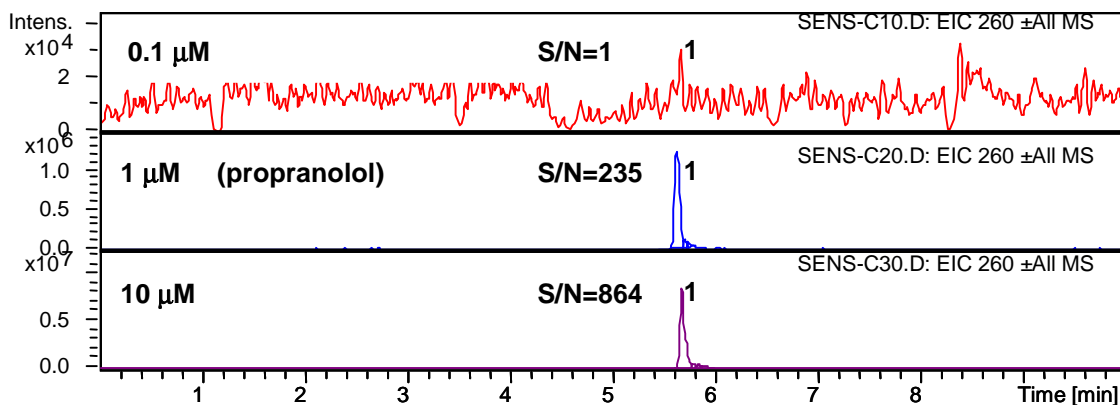
UV working concentration: 20-100 μM

Sensitivity and reproducibility of pressure-assisted-CE/MS

Total ion chromatogram (TIC)

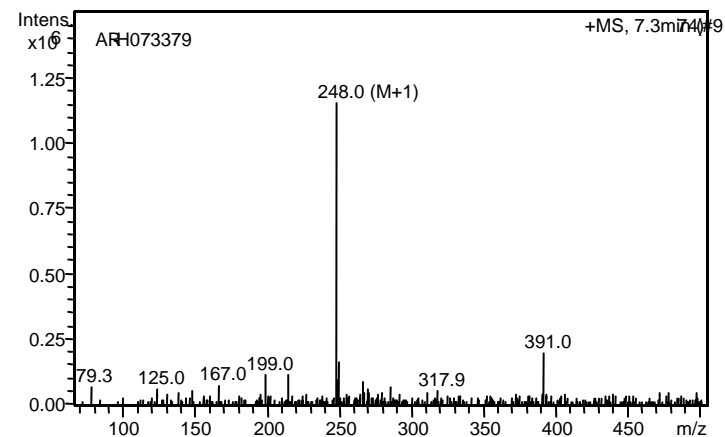
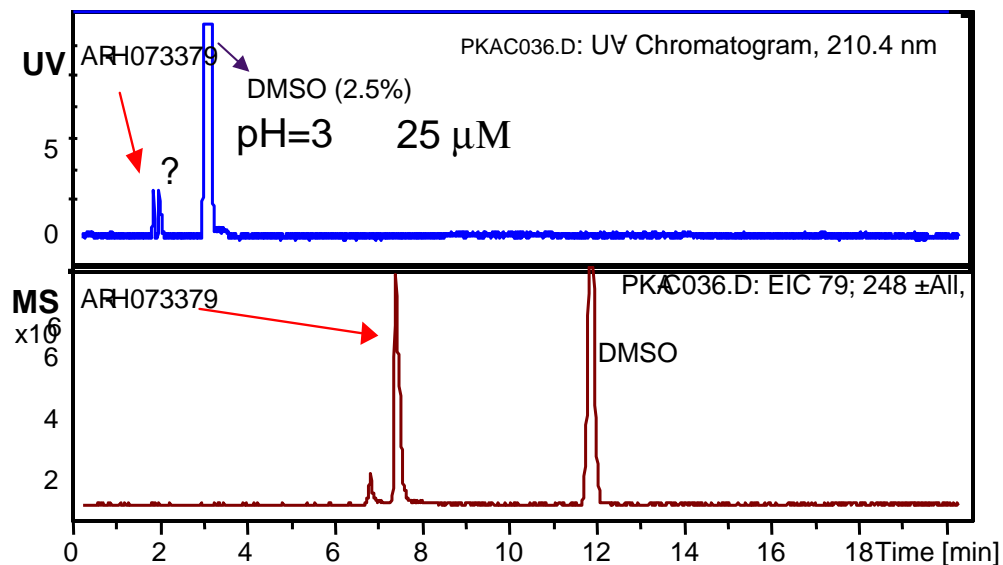


(EIC) and migration times after 30 runs



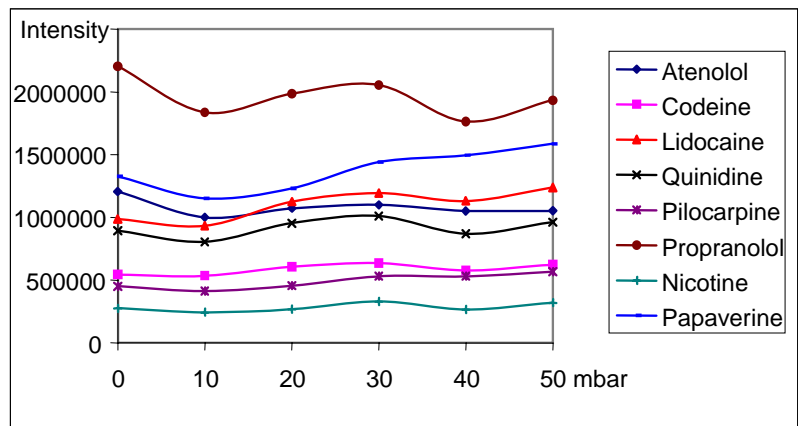
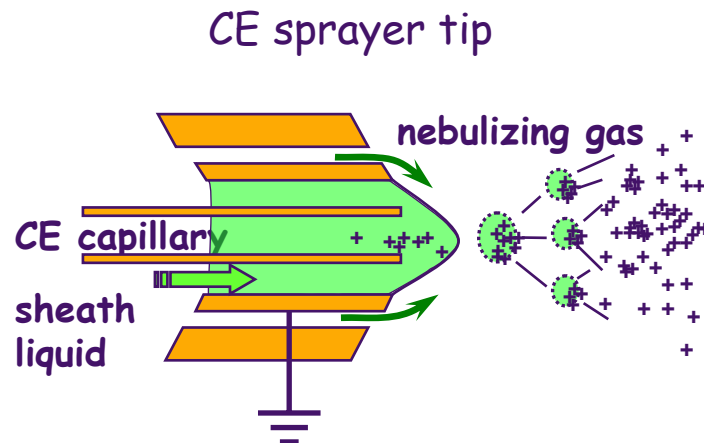
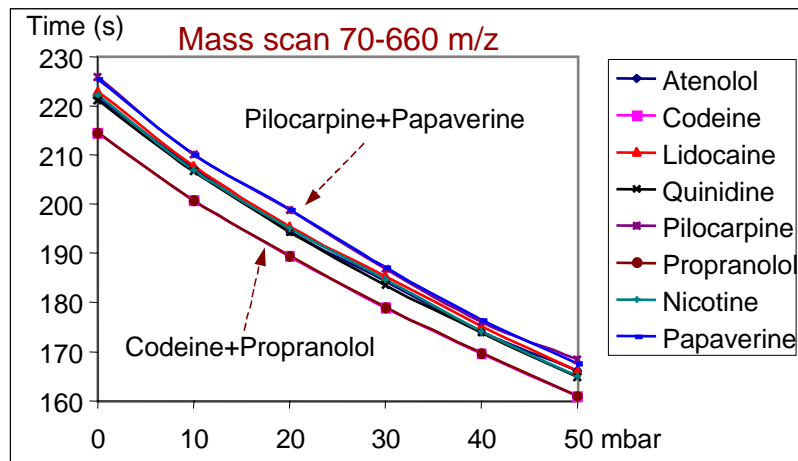
**Ammonium acetate
(pH=7) I=0.025;
pressure, 40 mbar**

Sensitive and selective MS detection over UV



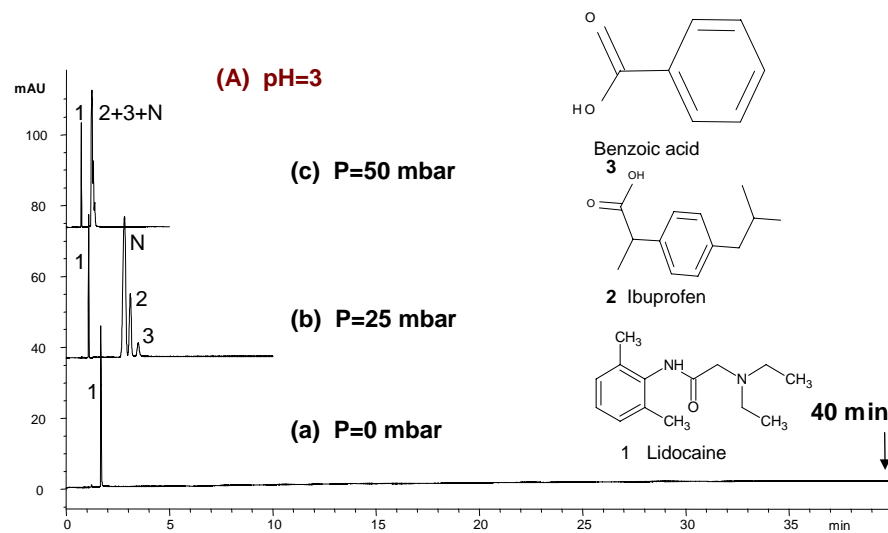
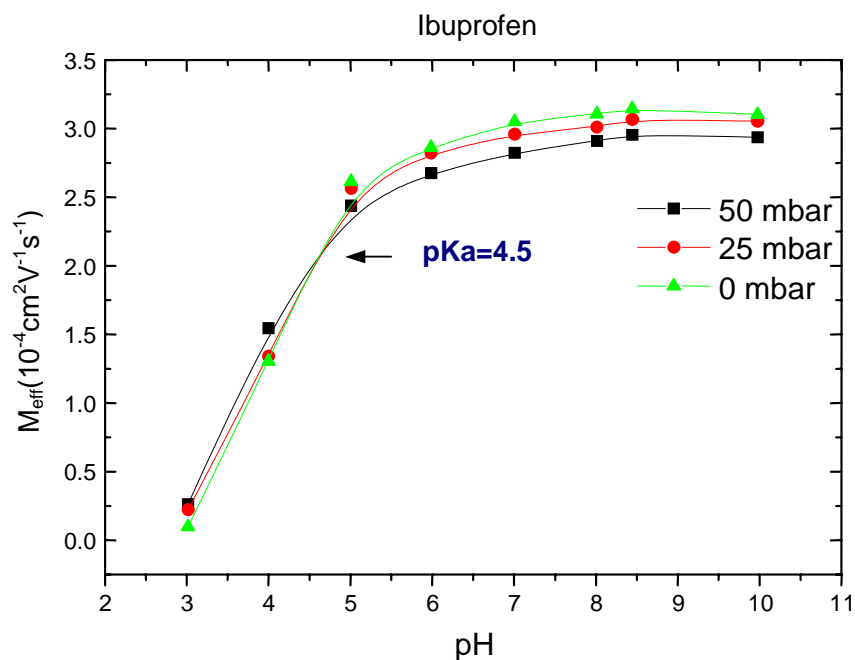
On-line tandem UV and MS detection

Effects of pressure on migration times/sensitivity



- Pressure reduced migration times
- Negligible signal suppression
- Improved RSD of effective mobility
- High throughput capacity

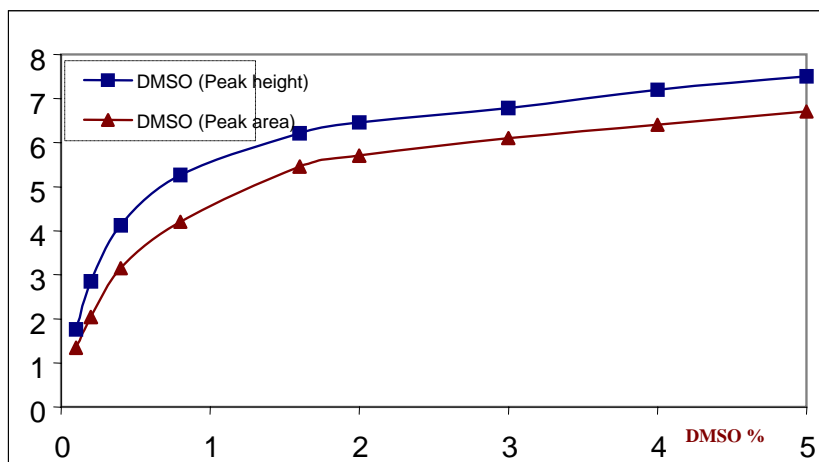
Effects of pressure on effective mobility



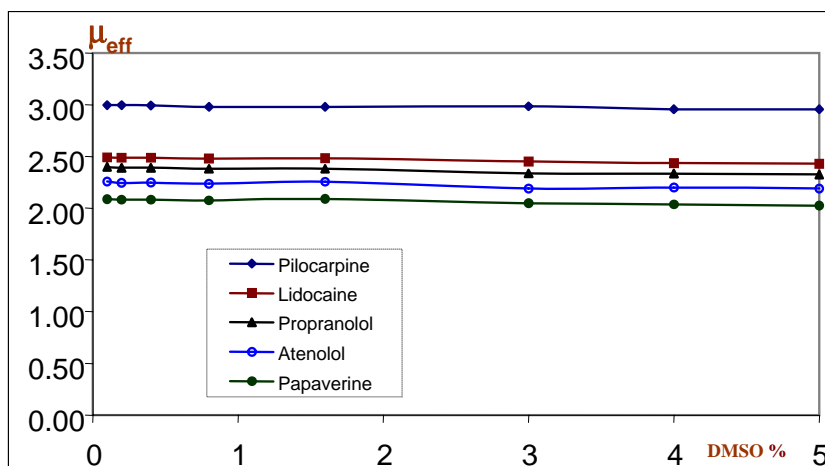
- Effective mobility shift around pKa (an interesting observation)
- Mobility shift caused by pressure doesn't affect pKa values.

H. Wan, A. Holmén, M. Någård, W. Lindberg, *J. Chromatogr. A.* 979 (2002) 369.

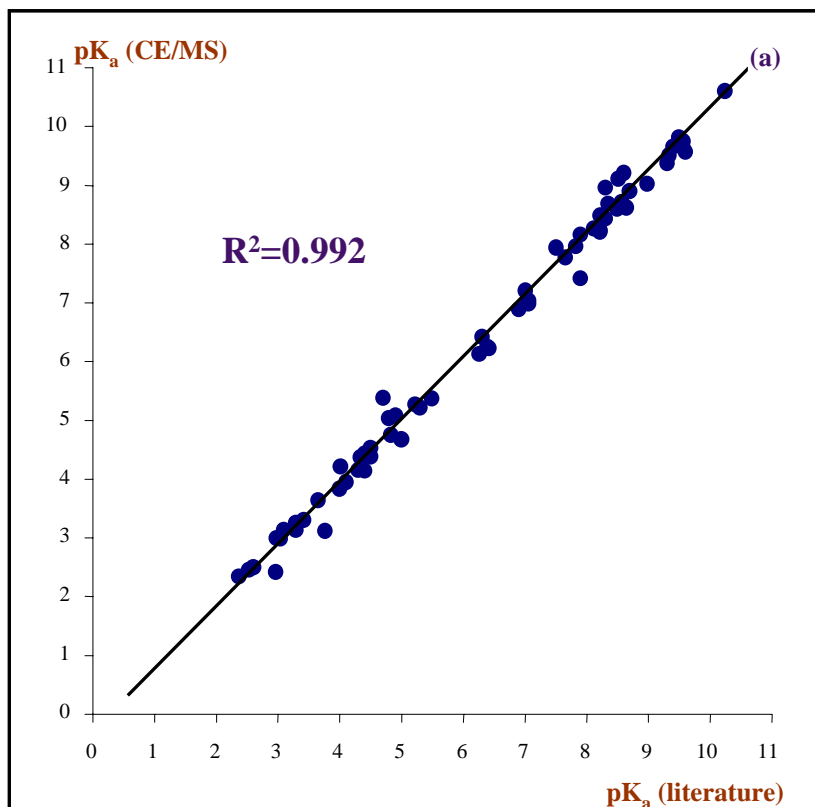
How many compounds can be pooled by CE/MS?



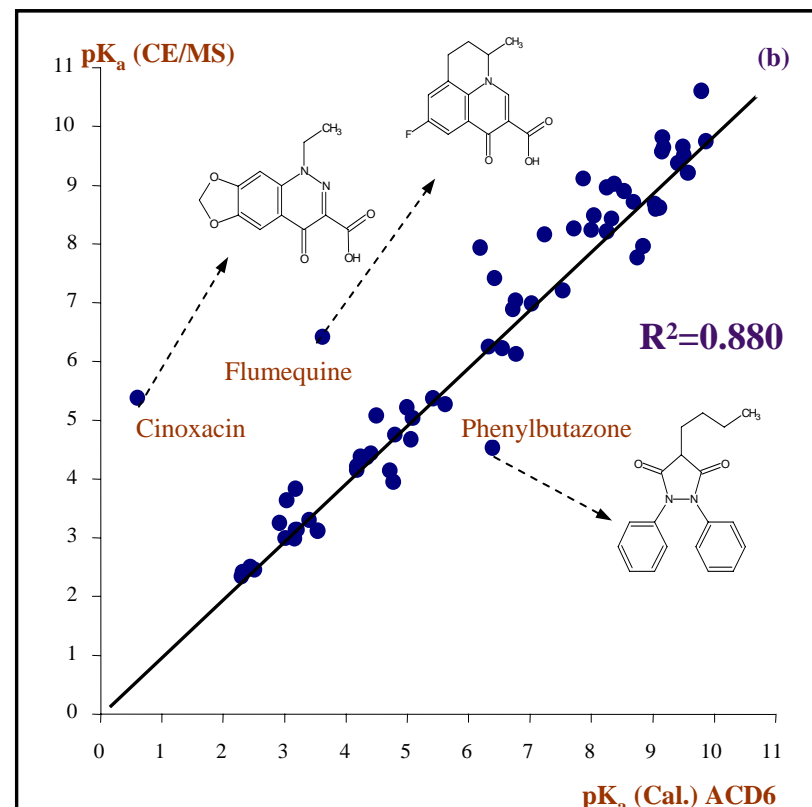
- DMSO < 5% in sample
- Constant mobility
- High MS resolution
- More than 150 cpds/sequence



Comparison of measured pKa and lit./predicted values

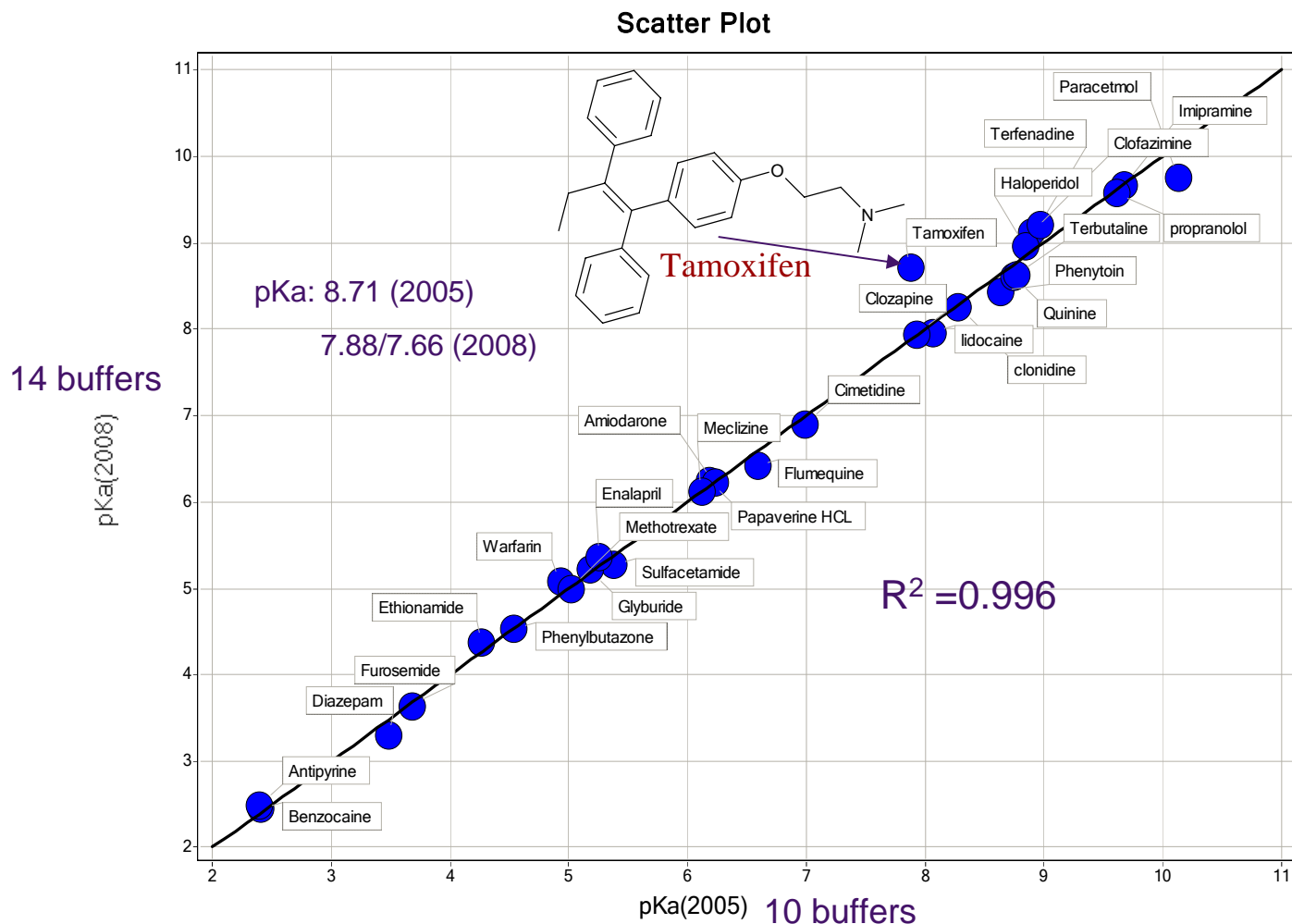


Poor prediction for conjugated structures

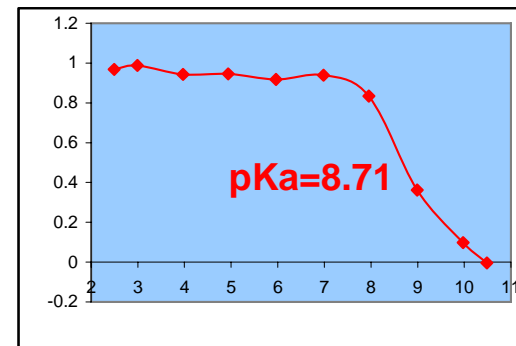
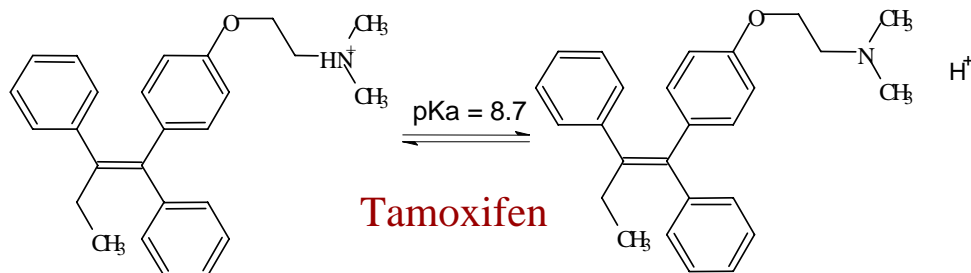


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Rapid Commun. Mass Spectrom. 17 (2003) 2639.

Long-term validation of CE/MS pKa (2005-2008)

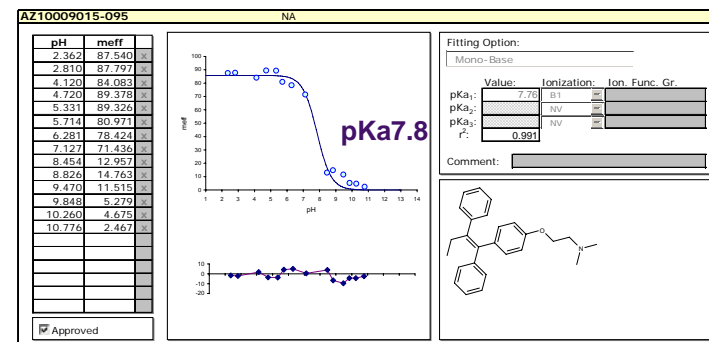


An example of poorly soluble compound



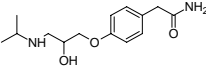
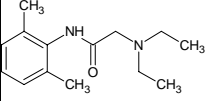
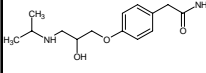
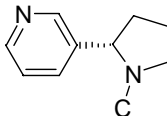
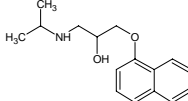
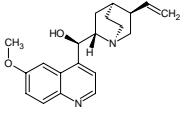
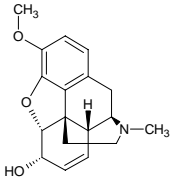
1. Ionization not close to chromophore
2. Low solubility 0.45 μM (pH 7.4), LogD=6.5

pKa (DPAS): no
 pKa (GlpKa): co-solvent, precipitation ?
 pKa (CE/MS): 8.71 (2005), 7.88/7.76 (2008)
 (ref: 7.6)



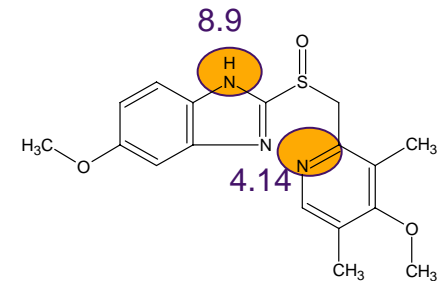
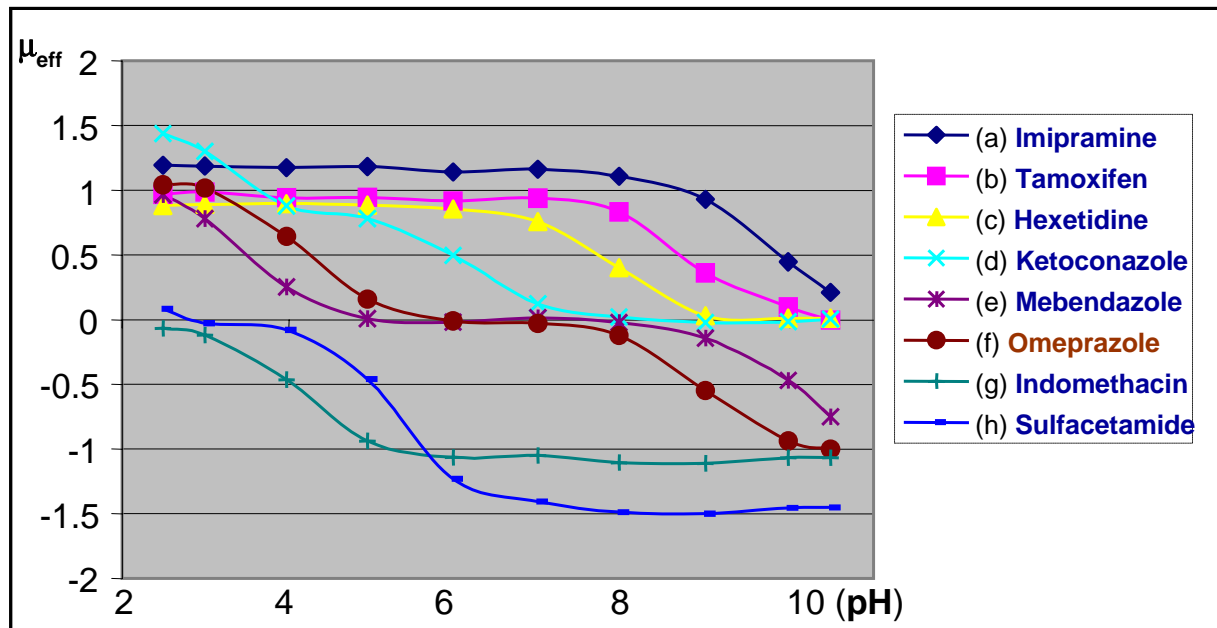
Ref. (Karl Box et al., Anal. Chem., 2003, 75, 883-892).

Long-term reproducibility of QC (2005-2008)

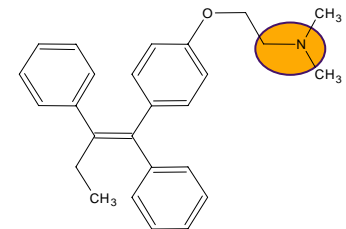
QC	AZ_1	AZ_2	AZ_3	AZ_4	AZ_5	AZ_6	AZ_7
Compound	Atenolol	Lidocaine	Papaverine	Nicotine	Propranolol	Quinine	Codeine
Structure							
pKa(B1/B2) Mean (n=50)	9.73	8.16	6.05	8.45(3.12)	9.67	8.88(4.00)	8.45
SD	0.09	0.09	0.11	0.10(0.27)	0.08	0.10(0.19)	0.08
Ref.	9.58	7.90	6.39/5.95	8.12(3.12)	9.50	8.50(4.1) 8.90 8.54	8.21 8.30
CE/UV	9.61	7.92	6.38	8.02(3.10)	9.49	8.39(4.14)	7.97

- Seven compounds as on-line QC for pKa screening (single measurement)
- Reproducibility <0.2 pKa units

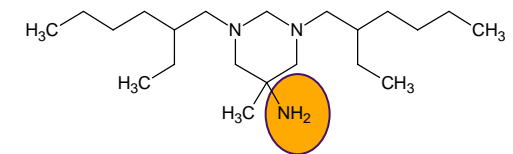
Examples of determination of poorly soluble compounds



(f) Omeprazole



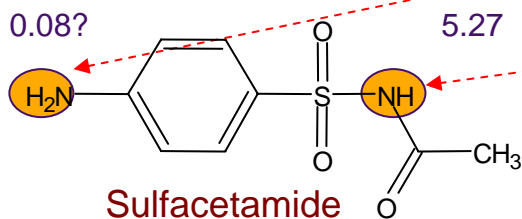
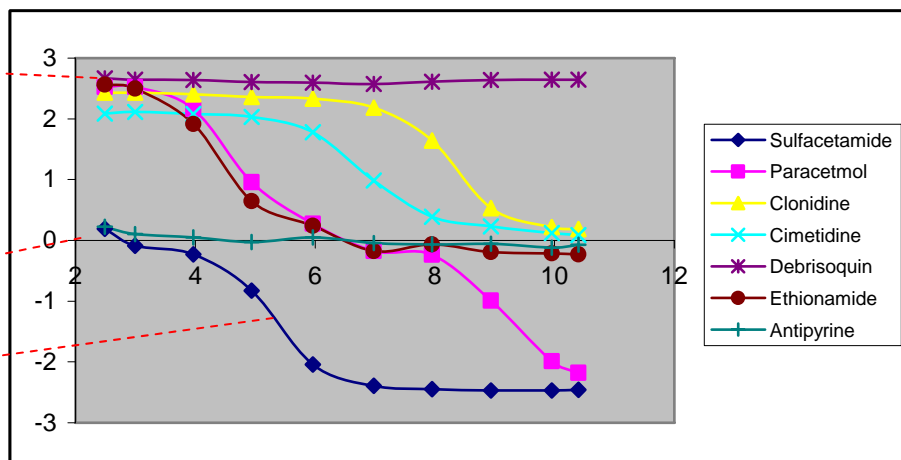
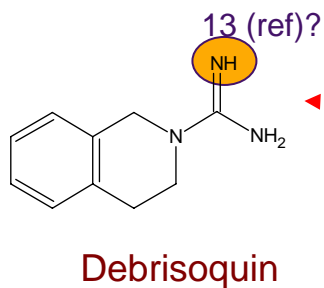
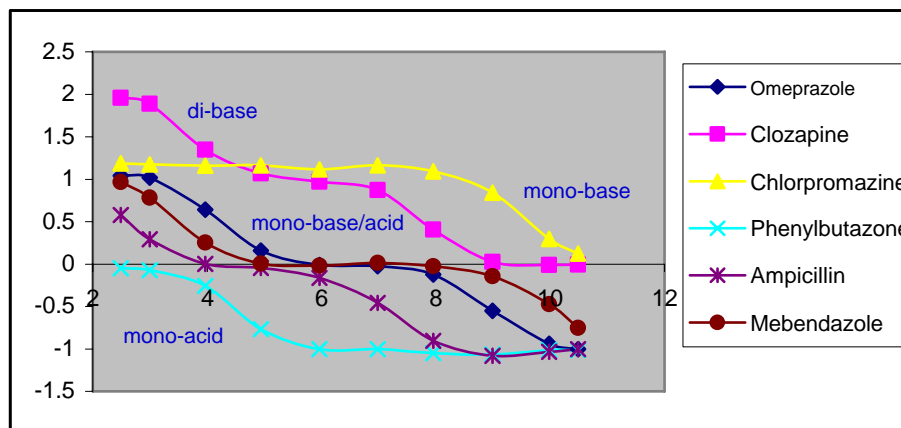
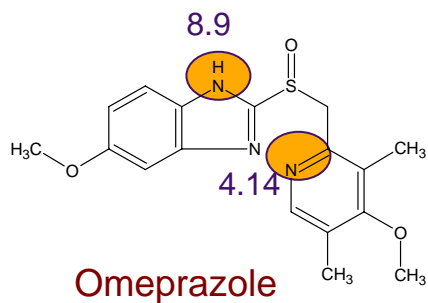
(b) Tamoxifen



(c) Hexetidine

- Possible to measure pKa with solubility $< 1 \mu\text{M}$
- $\text{pKa} = 50\% \text{ ionization} \leftrightarrow (\text{max. mobility})/2$
- Mobility \approx charge/mass

pKa fitting example pH 2.5-10.5/10 buffers(2.3-10.8/14 buffers)



Summary

- Pressure-assisted CE/MS for pKa:
 - High throughput screening pKa from 2.3 to 10.8
 - Reproducibility and accuracy of pKa \pm 0.2 units
 - Insensitive to compound purity, requiring minute sample
 - Concn: (1-10 μ M) beneficial for poorly soluble cpds
 - Independent on type of buffer, capillary length, DMSO concn.
 - Provide charge and structure/stability information (MS)

Acknowledgements

- Anders Holmén, Mats Någård, Walter Lindberg, Yudong Wang, Marie Englund, Richard Thompson, Johan Ulander

H. Wan, A. Holmén, M. Någård, W. Lindberg, J. Chromatogr. A. 979 (2002) 369.

H. Wan, A. Holmén, Y. Wang, W. Lindberg, M. Friberg, M. Någård, R. Thompson, Rapid Commun. Mass Spectrom. 17 (2003) 2639.

H. Wan, R. Thompson; Drug Discovery Today, Technologies, 2(2005), 171.

H. Wan, J. Ulander, Expert Opinion on Drug Metabolism & Toxicology, 2 (2006), 1389.

- Eva Emanuelsson (validation), Fredrik Bergström (new pKa-fit template test)
- Eva Thorin (Activitybase/Dixy group), Michael Wirth Färdigh (automation)
- Zhiping You (new pKa-fit program), AZ Boston
- Lead generation, Physical Chemistry group, AZ Mölndal