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

Hong Wan

Lead Generation, DMPK and Physical Chemistry
AstraZeneca R&D Mölndal
SE-431 83 Mölndal. SWEDEN
e-mail: hong.wan@astrazeneca.com

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

AstraZeneca

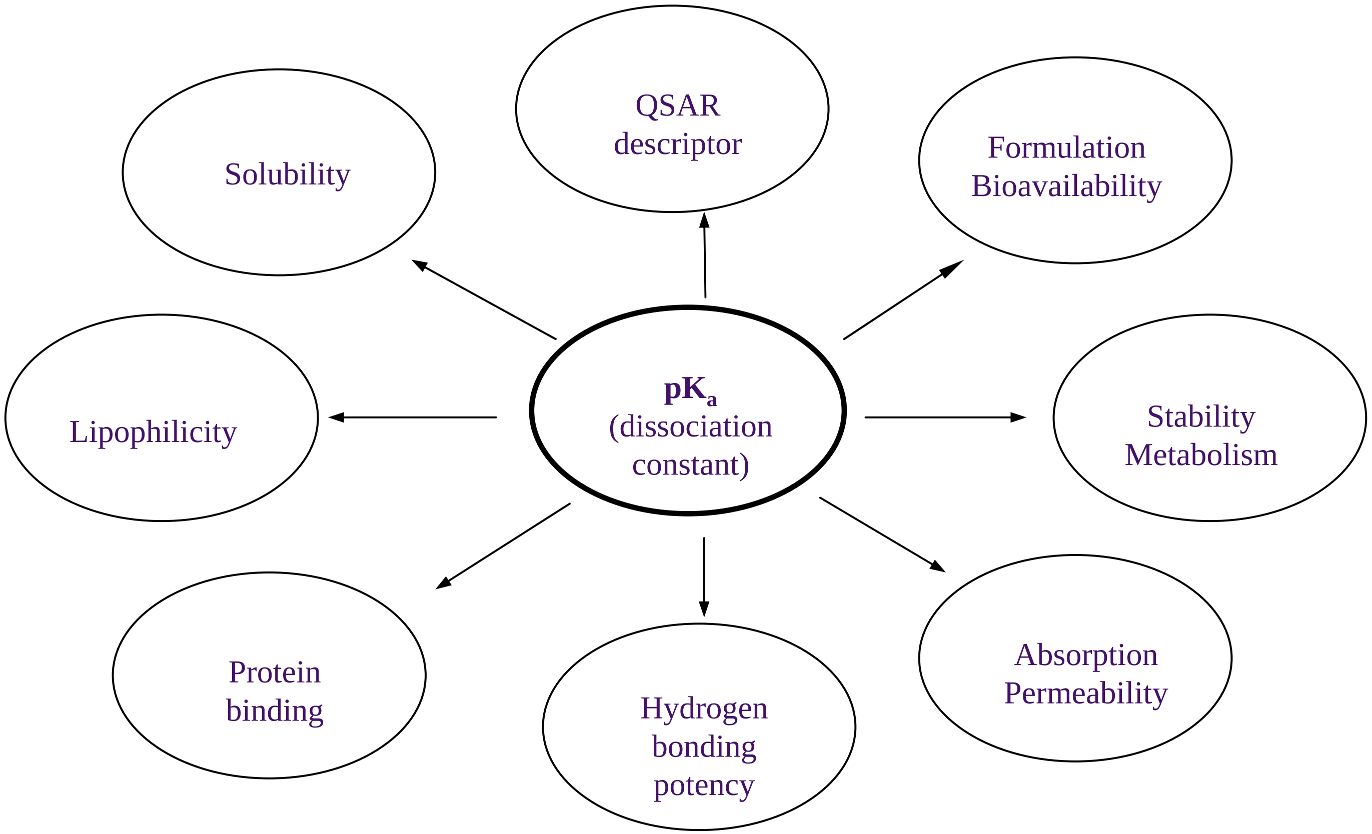
Outline

- 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

AstraZeneca

Screening for pKa, why?

- Info about change in charge of molecule……



AstraZeneca

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.

AstraZeneca

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	< ± 0.2	± 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

AstraZeneca

Principle of pKa determination by CE

pKa ↔ 50% ionization

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

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

AstraZeneca

Equations used for pKa calculation

Ionizable type	Model equation
Monobase	$m_{\mathrm{eff}} = \dfrac{M_{\mathrm{b}} 10^{-\mathrm{pH}}}{10^{-\mathrm{p}K_{\mathrm{a}}} + 10^{-\mathrm{pH}}}$
Monoacid	$m_{\mathrm{eff}} = \dfrac{M_{\mathrm{a}} 10^{-\mathrm{p}K_{\mathrm{a}}}}{10^{-\mathrm{p}K_{\mathrm{a}}} + 10^{-\mathrm{pH}}}$
Dibase	$m_{\mathrm{eff}} = \dfrac{M_{\mathrm{b2}}[10^{-\mathrm{pH}}]^2 + M_{\mathrm{b1}} 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{pH}}}{[10^{-\mathrm{pH}}]^2 + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{pH}} + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}}}$
Diacid	$m_{\mathrm{eff}} = \dfrac{M_{\mathrm{a1}} 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{pH}} + M_{\mathrm{a2}} 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}}}{[10^{-\mathrm{pH}}]^2 + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{pH}} + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}}}$
Monoacidic monobasic ampholyte	$m_{\mathrm{eff}} = \dfrac{M_{\mathrm{b1}}[10^{-\mathrm{pH}}]^2 + M_{\mathrm{a1}} 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}}}{[10^{-\mathrm{pH}}]^2 + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{pH}} + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}}}$
Tribase	$m_{\mathrm{eff}} = \dfrac{M_{\mathrm{b3}}[10^{-\mathrm{pH}}]^3 + M_{\mathrm{b2}} 10^{-\mathrm{p}K_{\mathrm{a1}}}[10^{-\mathrm{pH}}]^2 + M_{\mathrm{b1}} 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{pH}}}{[10^{-\mathrm{pH}}]^3 + 10^{-\mathrm{p}K_{\mathrm{a1}}}[10^{-\mathrm{pH}}]^2 + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{pH}} + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{p}K_{\mathrm{a3}}}}$
Triacid	$m_{\mathrm{eff}} = \dfrac{M_{\mathrm{a1}} 10^{-\mathrm{p}K_{\mathrm{a1}}}[10^{-\mathrm{pH}}]^2 + M_{\mathrm{a2}} 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{pH}} + M_{\mathrm{a3}} 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{p}K_{\mathrm{a3}}}}{[10^{-\mathrm{pH}}]^3 + 10^{-\mathrm{p}K_{\mathrm{a1}}}[10^{-\mathrm{pH}}]^2 + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{pH}} + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{p}K_{\mathrm{a3}}}}$
Diacidic monobasic ampholyte	$m_{\mathrm{eff}} = \dfrac{M_{\mathrm{b1}}[10^{-\mathrm{pH}}]^3 + M_{\mathrm{a1}} 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{pH}} + M_{\mathrm{a2}} 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{p}K_{\mathrm{a3}}}}{[10^{-\mathrm{pH}}]^3 + 10^{-\mathrm{p}K_{\mathrm{a1}}}[10^{-\mathrm{pH}}]^2 + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{pH}} + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{p}K_{\mathrm{a3}}}}$
Monoacidic dibasic ampholyte	$m_{\mathrm{eff}} = \dfrac{M_{\mathrm{b2}}[10^{-\mathrm{pH}}]^3 + M_{\mathrm{b1}} 10^{-\mathrm{p}K_{\mathrm{a1}}}[10^{-\mathrm{pH}}]^2 + M_{\mathrm{a1}} 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{p}K_{\mathrm{a3}}}}{[10^{-\mathrm{pH}}]^3 + 10^{-\mathrm{p}K_{\mathrm{a1}}}[10^{-\mathrm{pH}}]^2 + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{pH}} + 10^{-\mathrm{p}K_{\mathrm{a1}}} 10^{-\mathrm{p}K_{\mathrm{a2}}} 10^{-\mathrm{p}K_{\mathrm{a3}}}}$

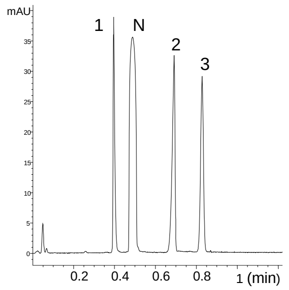
Graphs and table from Miller, *Electrophoresis* 2002, 23, 2833

High-throughput pKa screening by CE/MS

10-14 different pH buffers → Constant ionic strengths

↓

CE separation → ***Pressure***



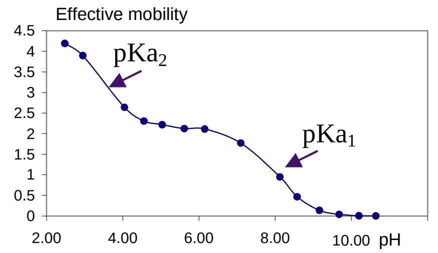
UV	MS
non-volatile buffers	Volatile buffers
(Single compound)	(Pooled compounds)
20-100 µM	

Mobility calculation →

$$m_{eff} = m_{obs} - m_{eof} = \frac{L_{tot} \cdot L_{eff}}{V} \cdot \left(\frac{1}{t_{obs}} - \frac{1}{t_{eof}} \right)$$

↓

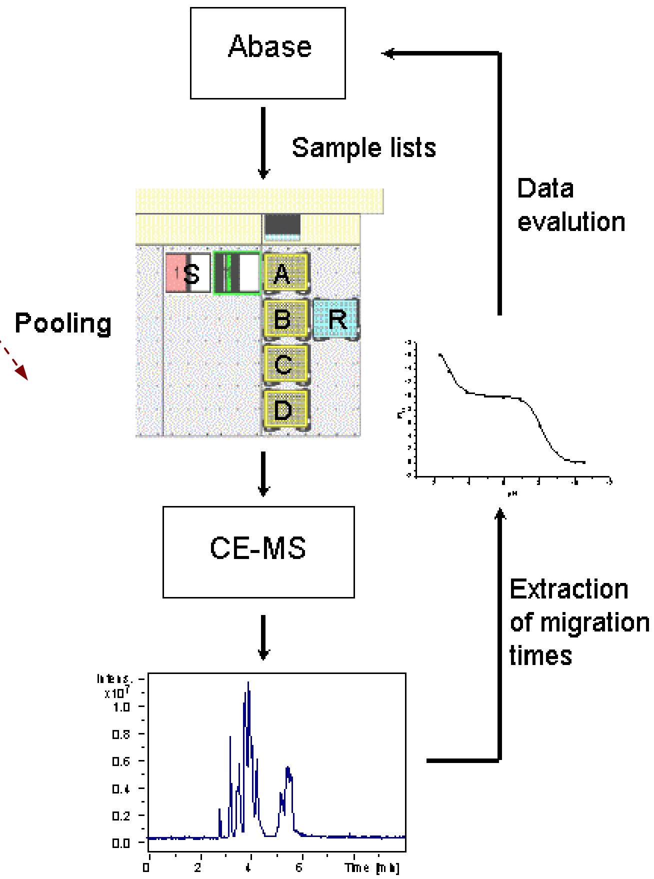
pK$_a$ evaluation → (fit-pK$_a$)



AstraZeneca

Sample pooling and data analysis flow scheme

Reference stand.



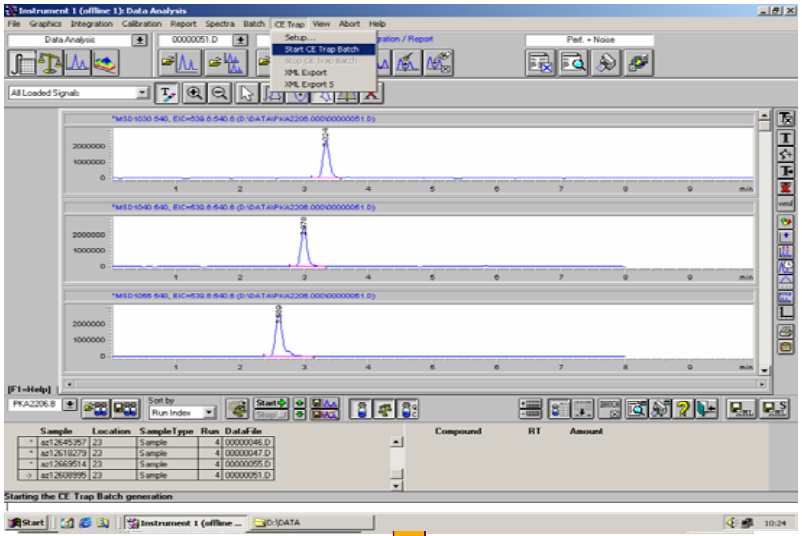
2003 manual data analysis

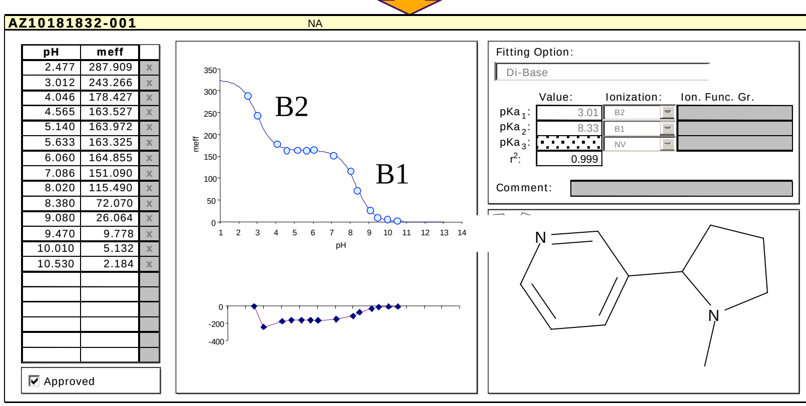
H. Wan, A. Holmén, Y. Wang, W. Lindberg, M. Englund, M. Någård, R. Thompson,

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

AstraZeneca

Current pKa screening - integrated & automated assay





Generate sample run list (Abase)

↓

Automated sample pooling (mass)

↓

CE/MS

↓

Automated migration time conversion

↓

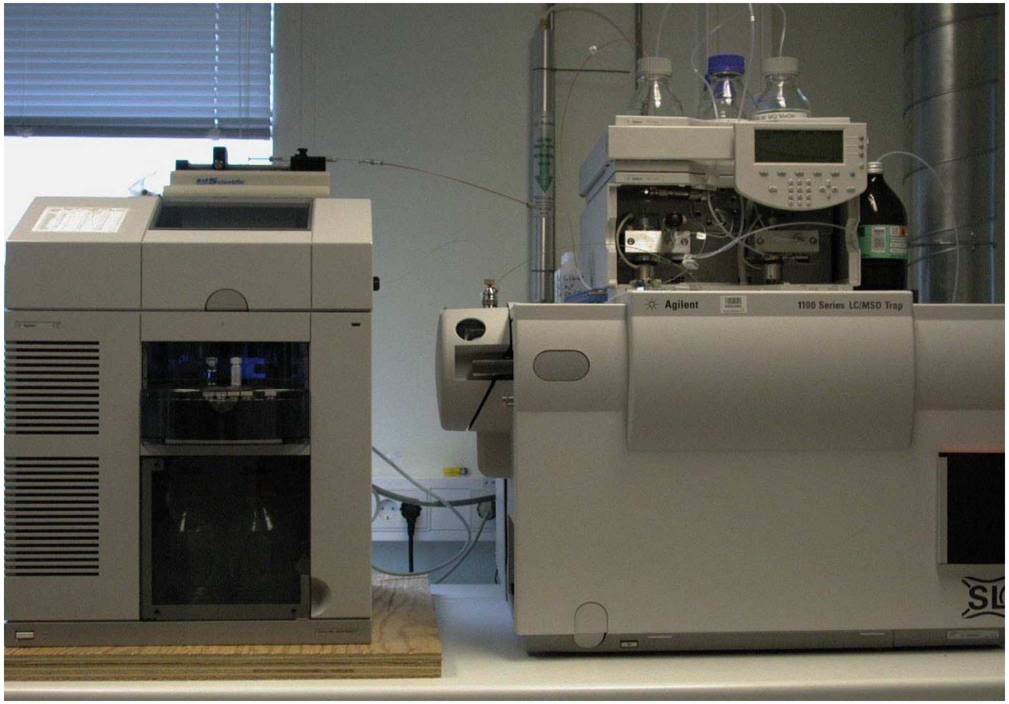
pKa evaluation

↓

pKa reported to database

AstraZeneca

CE/MS instrumentation



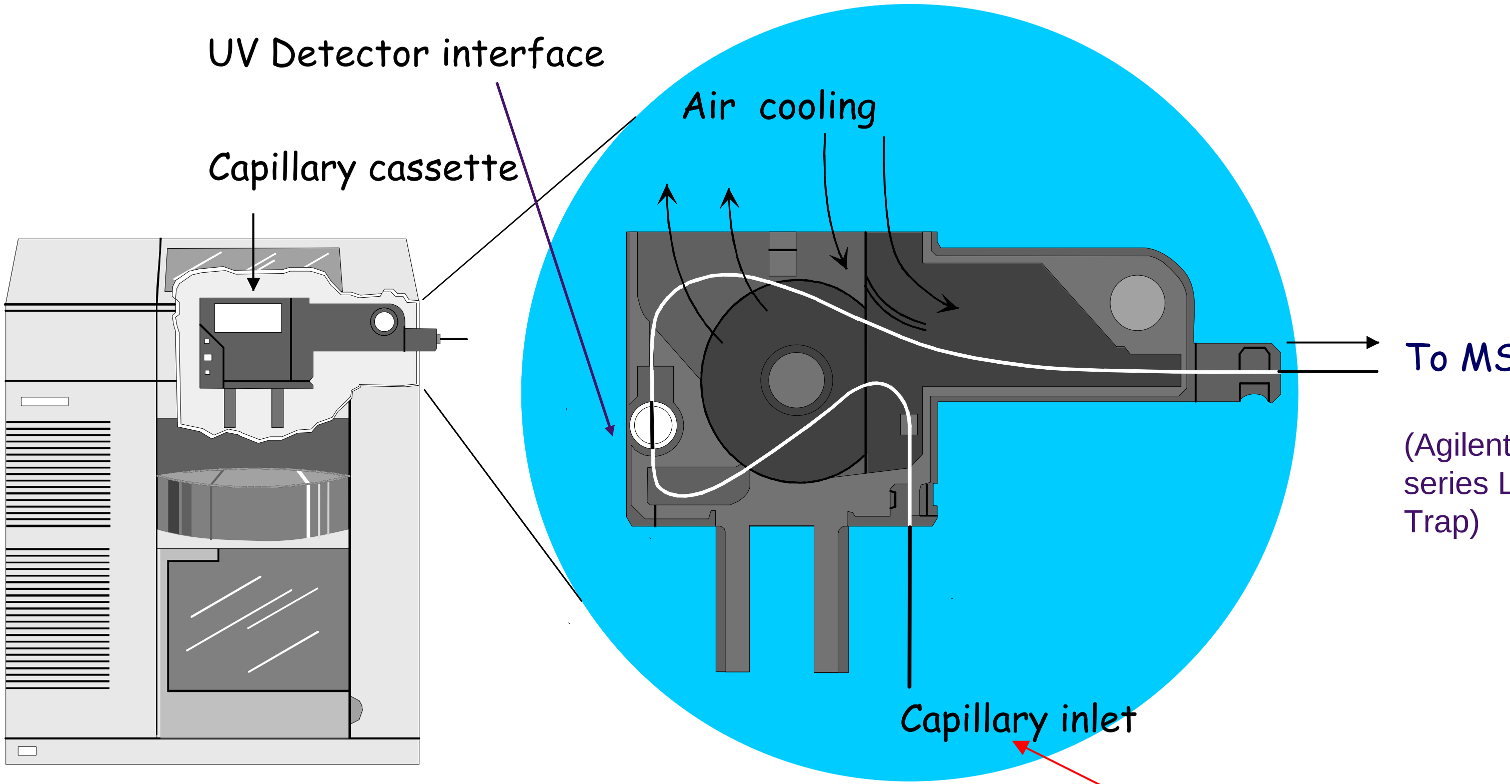
- Higher initial investment
- Long-term savings

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).

AstraZeneca

On-line CE/UV/MS experimental setup



(Agilent 1100 series LC/MSD Trap)

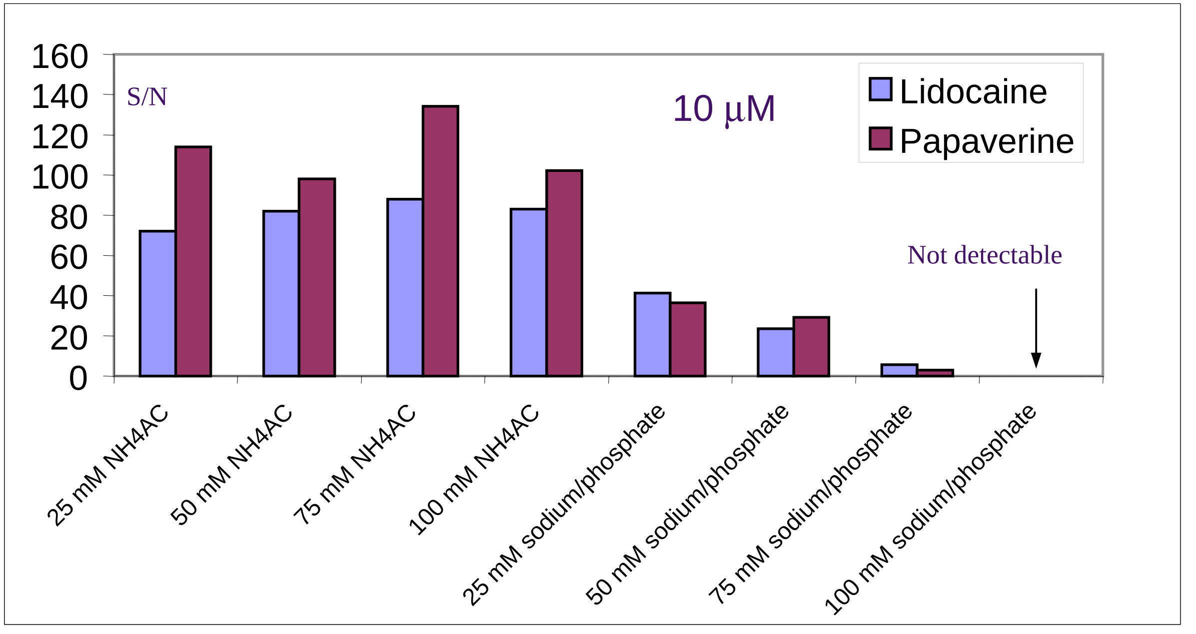
UV @ 21.6 cm
MS @ > 75 cm
MS w/o UV @ 55 cm

Pressure (40-50 mbar)

Courtesy from Agilent (with small modifications)

AstraZeneca

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

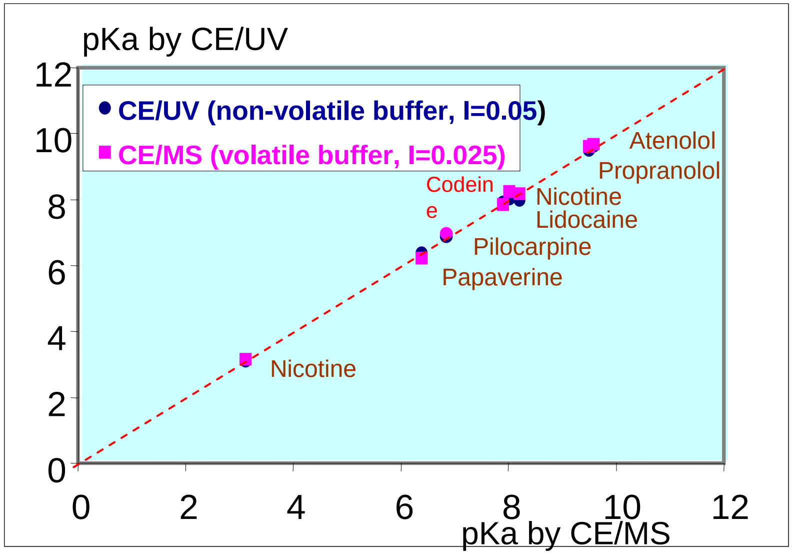


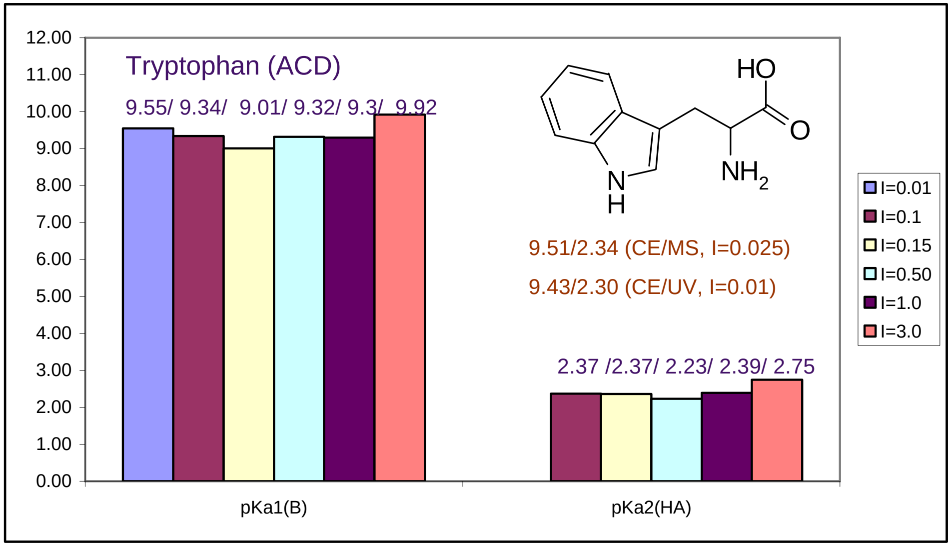
- Non-volatile buffer decreased signals at higher concentration (ion suppression)
- Ion source contamination by non-volatile buffer

AstraZeneca

Effects of buffer type and ionic strength on pKa

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

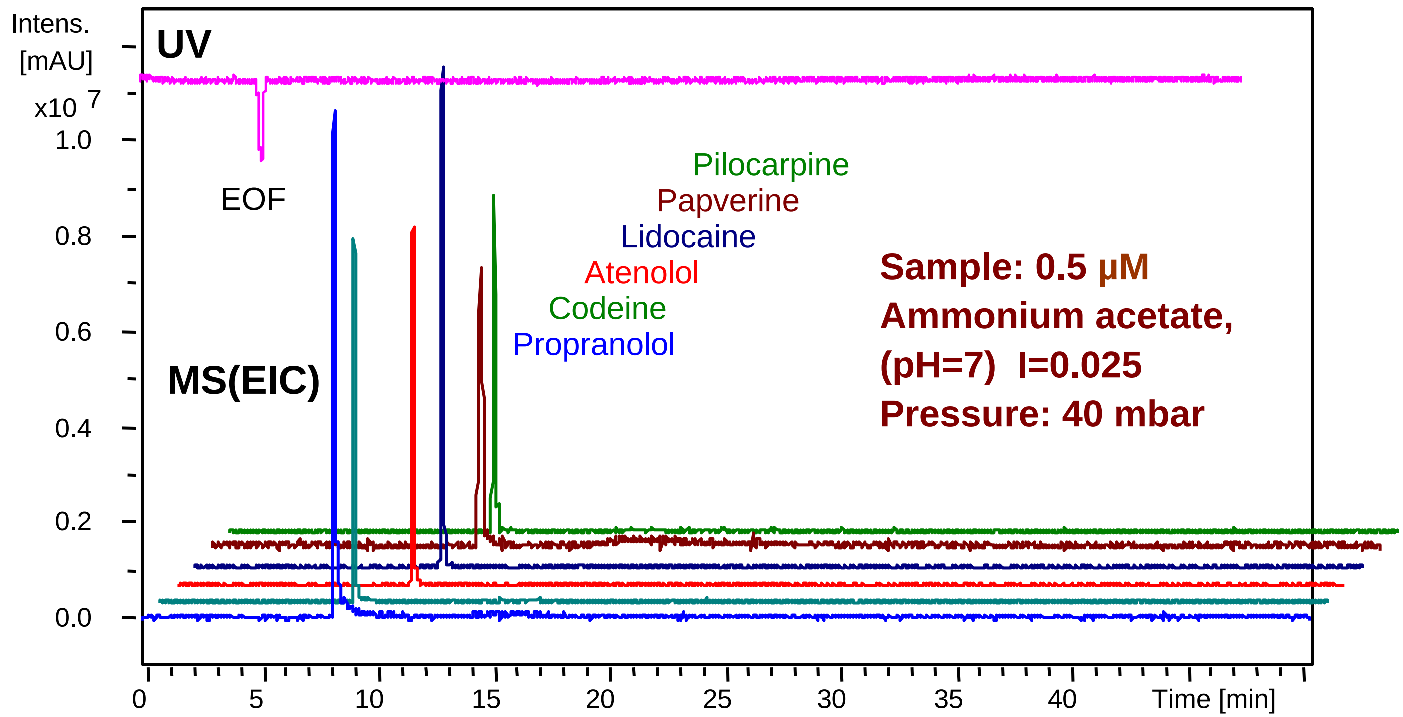




- Titration method uses physiological buffer with ionic strength at 0.15 M.
- Ionic strengths (0.025 - 0.15 M) have a small effect on pKa (Δ pKa=0.064)

AstraZeneca

Comparison of UV and MS (Ion trap) sensitivity

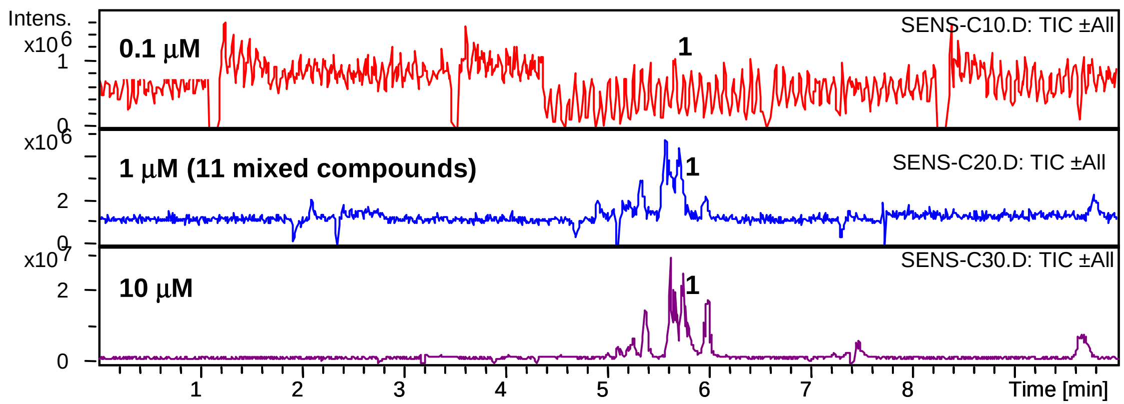


UV working concentration: 20-100 µM

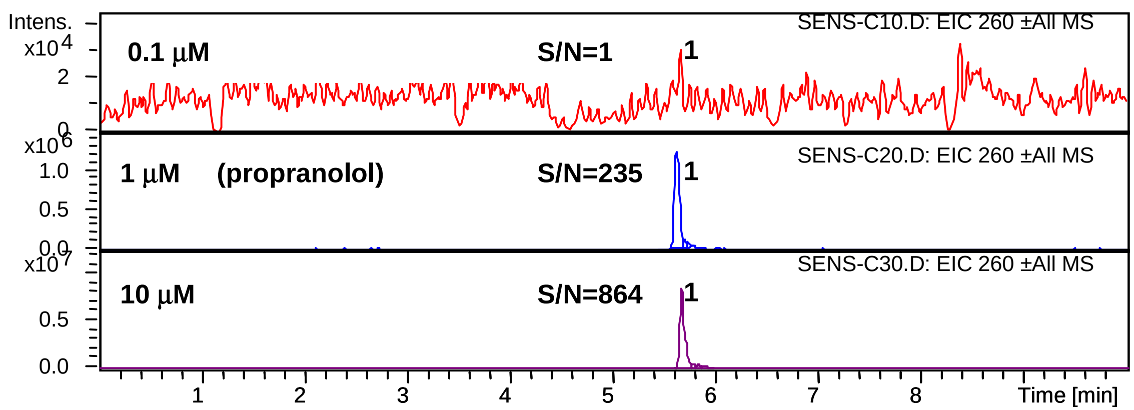
AstraZeneca

Sensitivity and reproducibility of pressure-assisted-CE/MS

Total ion chromatogram (TIC)



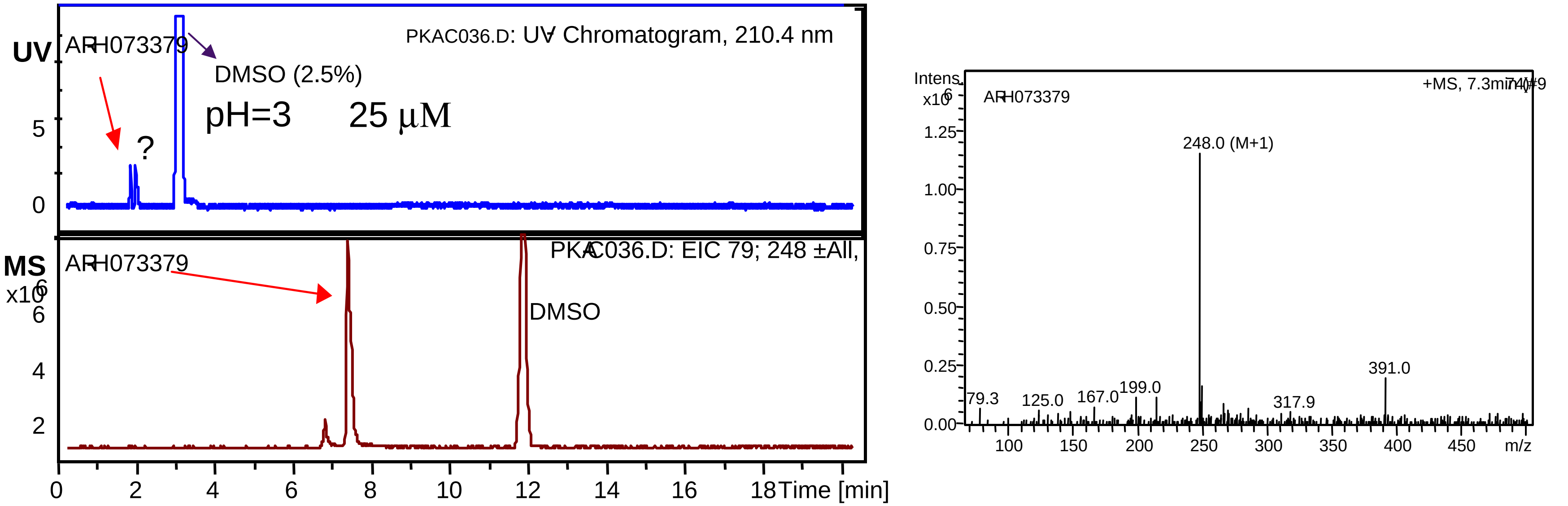
(EIC) and migration times after 30 runs



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

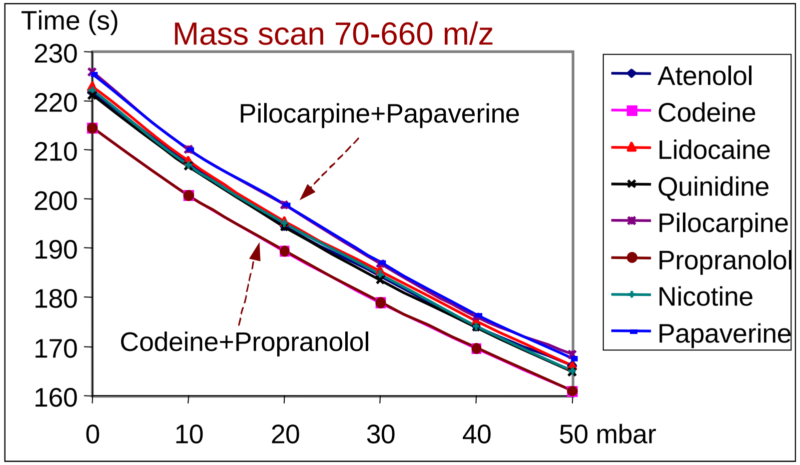
AstraZeneca

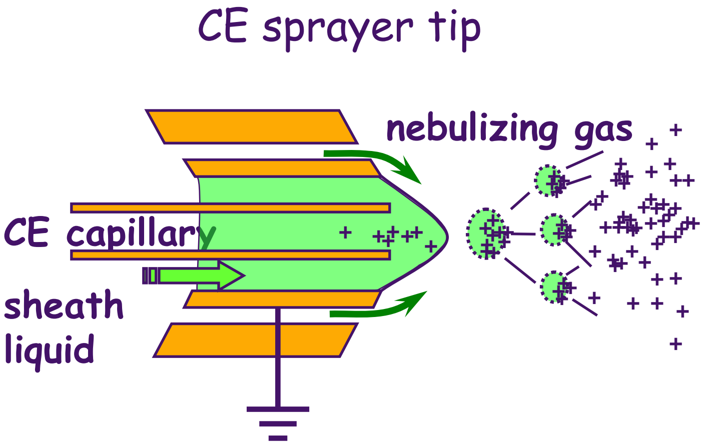
Sensitive and selective MS detection over UV

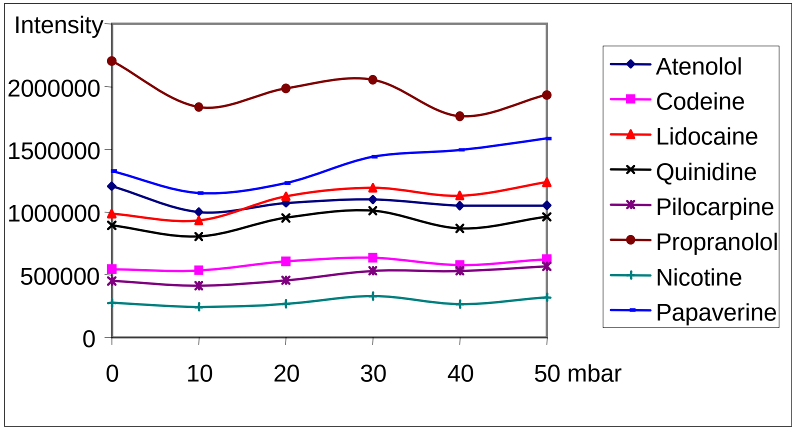


On-line tandem UV and MS detection

Effects of pressure on migration times/sensitivity



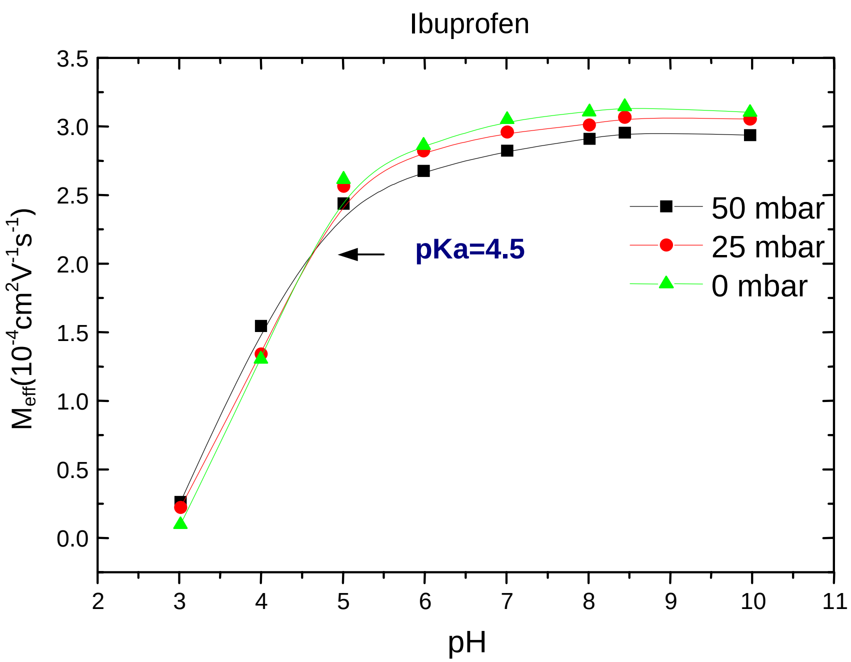


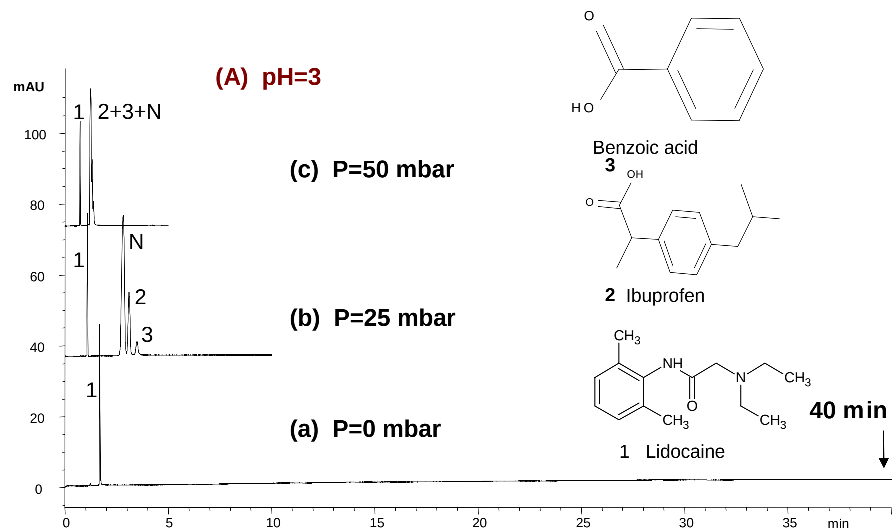


- Pressure reduced migration times
- Negligible signal suppression
- Improved RSD of effective mobility
- High throughput capacity

AstraZeneca

Effects of pressure on effective mobility



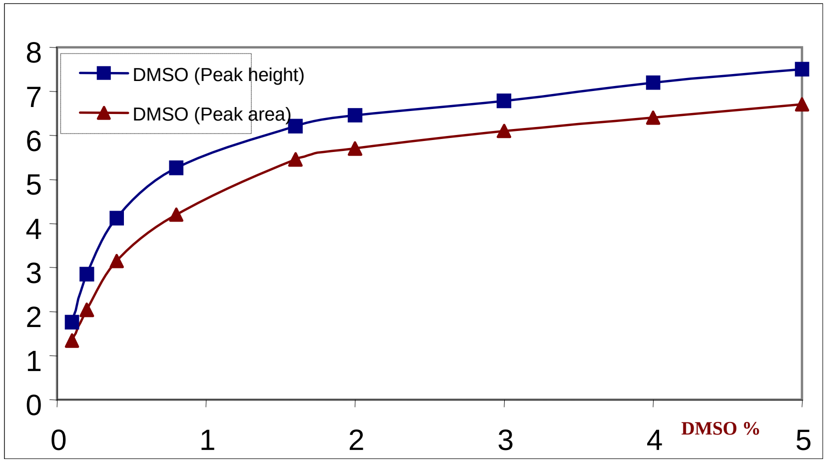


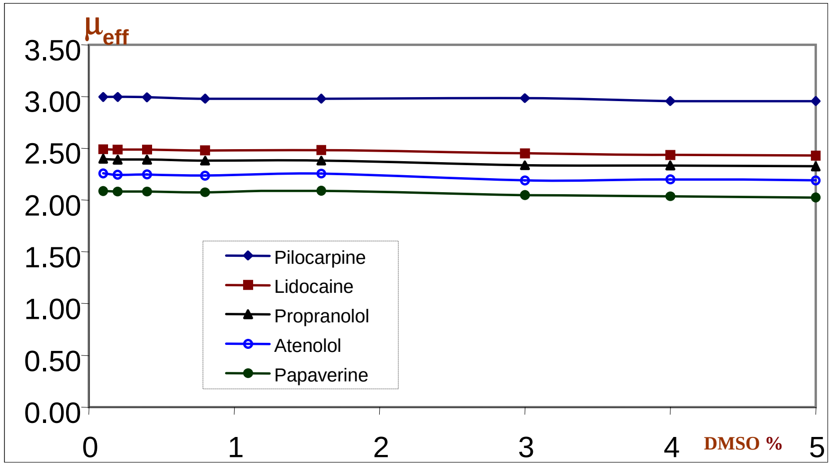
- Effective mobility shift around pKa (an interesting observation)
- Mobility shift caused by pressure doesn't affect pKa values.

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

AstraZeneca

How many compounds can be pooled by CE/MS?



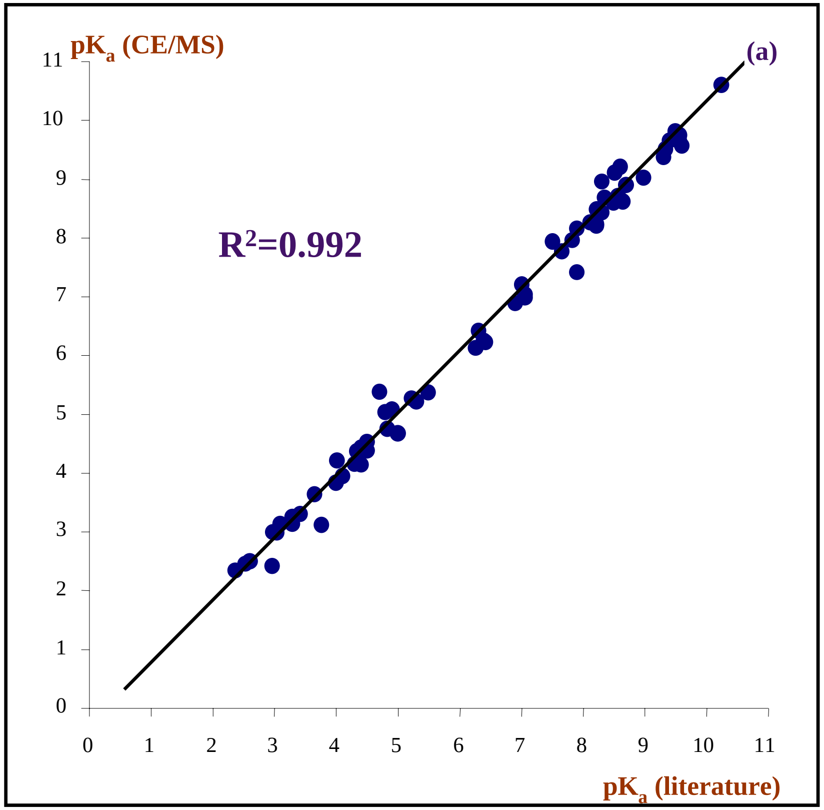


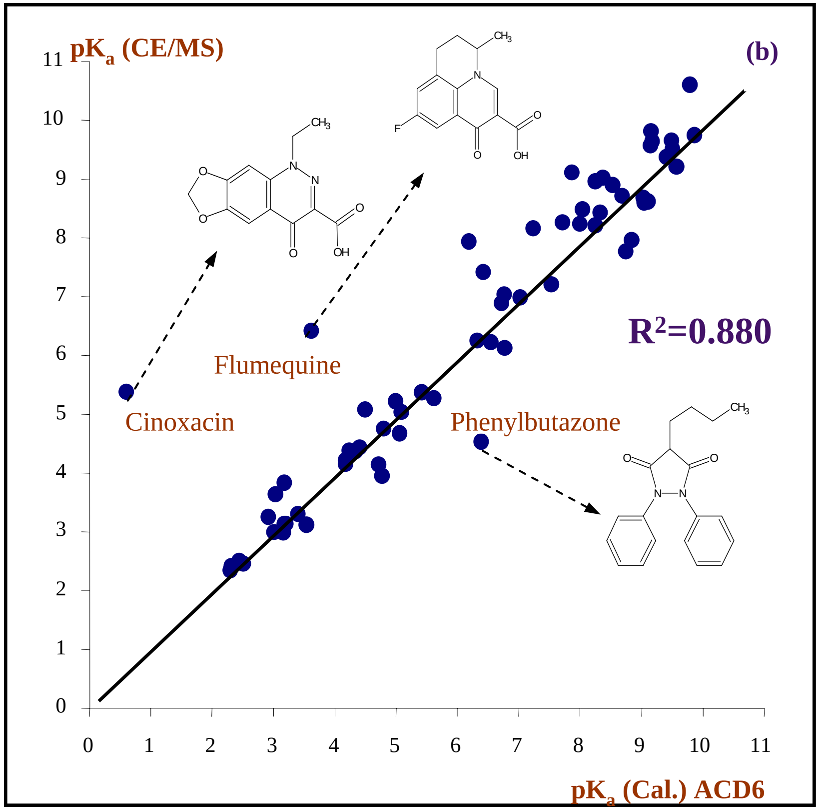
- DMSO < 5% in sample
- Constant mobility
- High MS resolution
- More than 150 cpds/sequence

AstraZeneca

Comparison of measured pKa and lit./predicted values

Poor prediction for conjugated structures



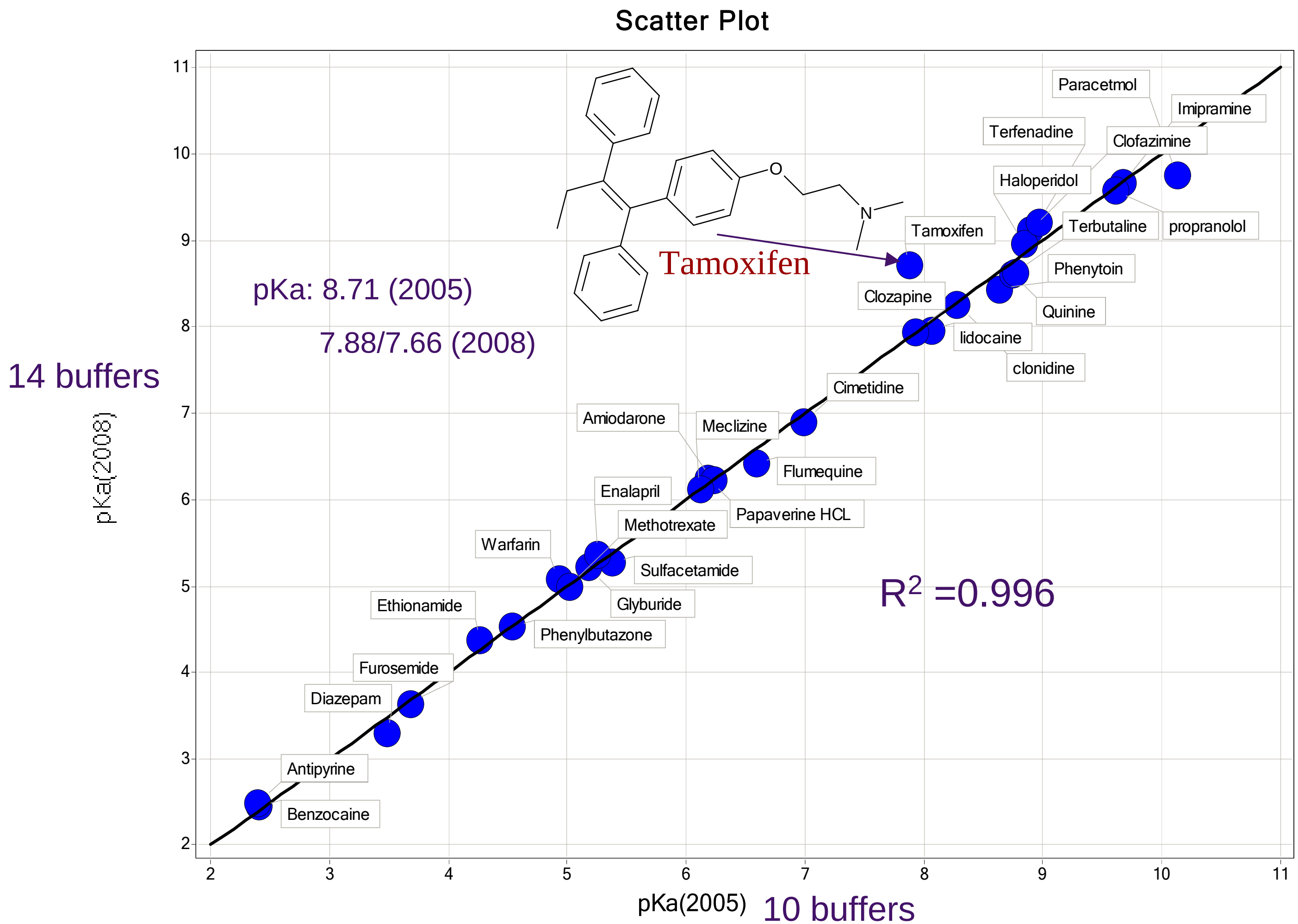


H. Wan, A. Holmén, Y. Wang, W. Lindberg, M. Englund, M. Någård, R. Thompson,

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

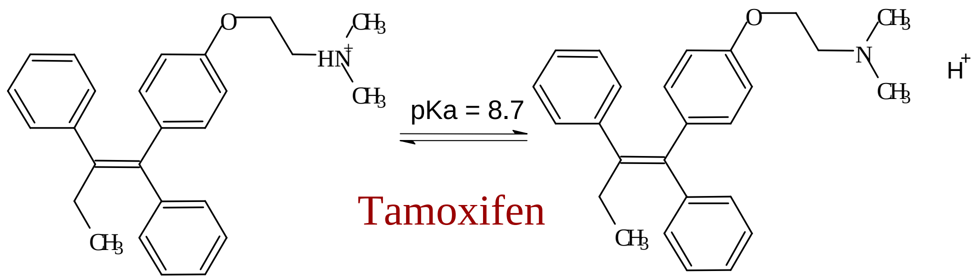
AstraZeneca

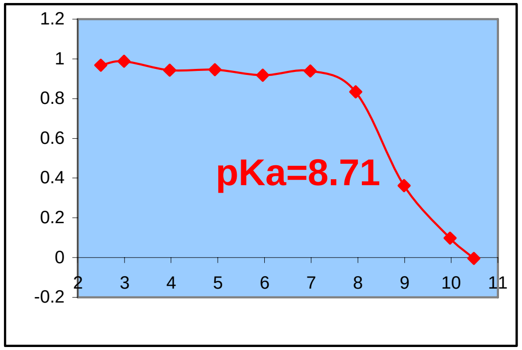
Long-term validation of CE/MS pKa (2005-2008)

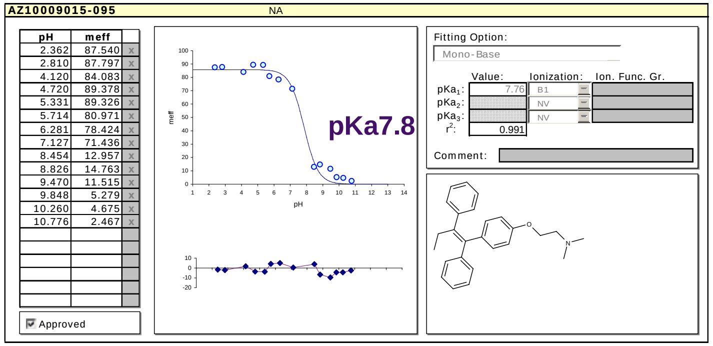


AstraZeneca

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

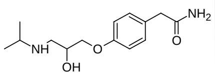
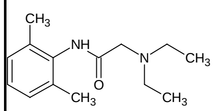
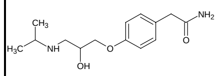
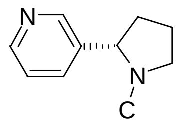
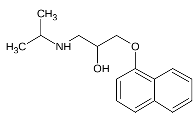
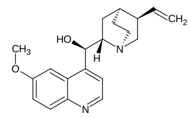
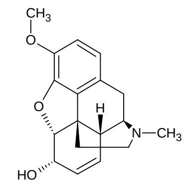
pKa (GlpKa): co-solvent, precipitation ?

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).

AstraZeneca

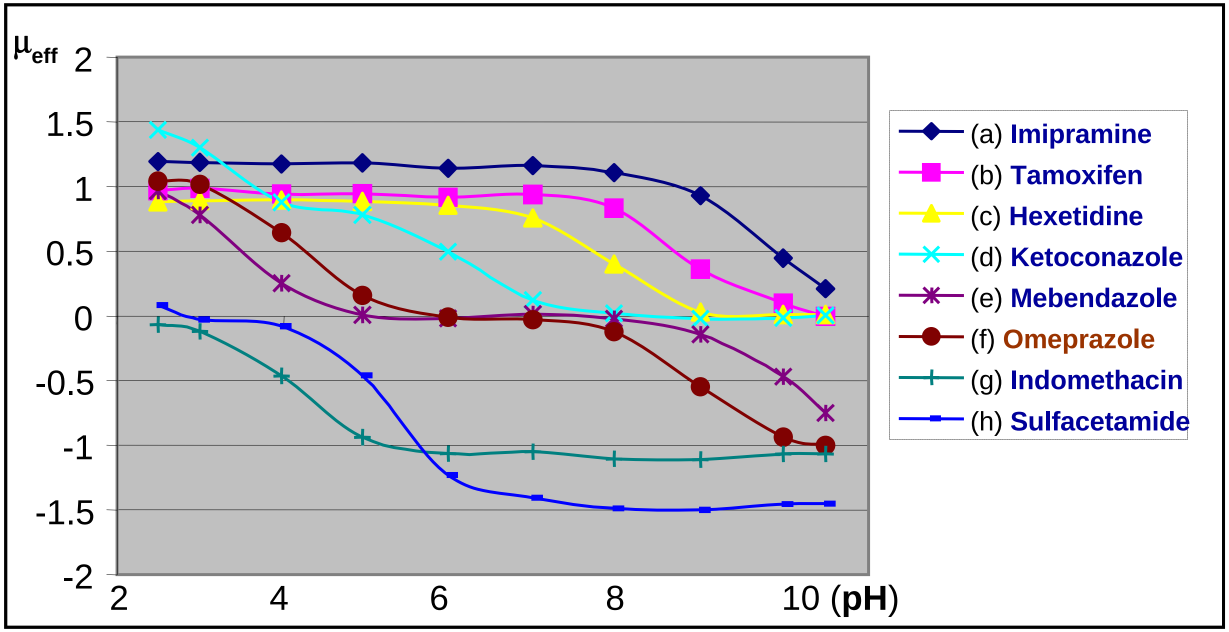
Long-term reproducibility of QC (2005-2008)

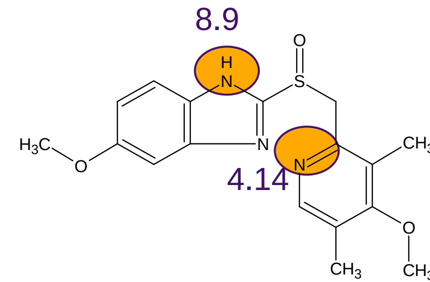
QC	AZ_1	AZ_2	AZ_3	AZ_4	AZ_5	AZ_6	AZ_7
Compound	Atenolol	Lidocaine	Papaverine	Nicotine	Propranolol	Quinine	Codeine
Structure							
pKa(B1/B2) Mean (n=50)	9.73	8.16	6.05	8.45(3.12)	9.67	8.88(4.00)	8.45
SD	0.09	0.09	0.11	0.10(0.27)	0.08	0.10(0.19)	0.08
Ref.	9.58	7.90	6.39/5.95	8.12(3.12)	9.50	8.50(4.1) 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

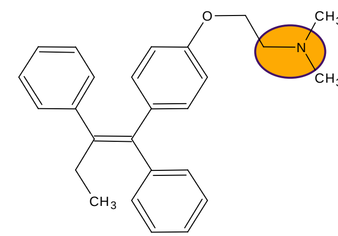
AstraZeneca

Examples of determination of poorly soluble compounds

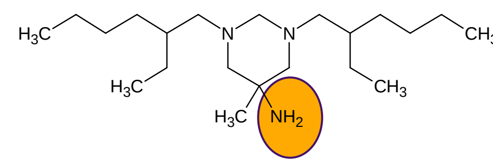




(f) Omeprazole



(b) Tamoxifen

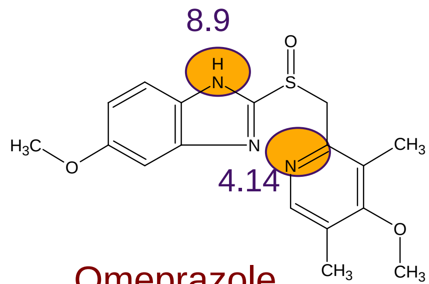


(c) Hexetidine

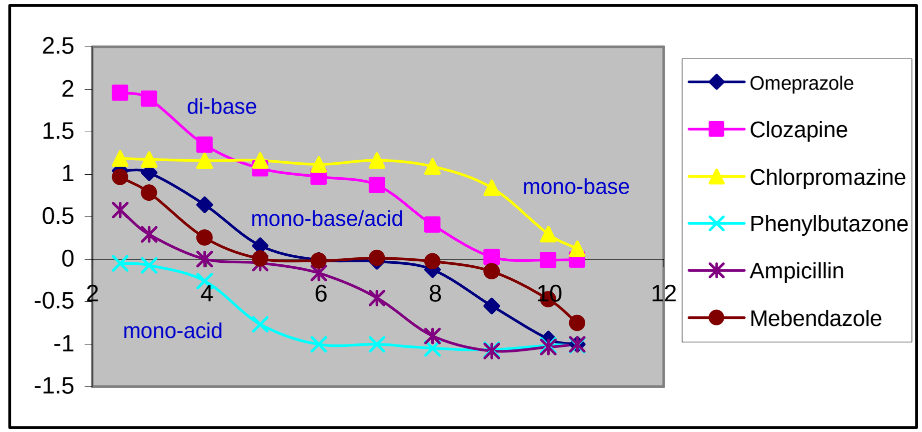
- Possible to measure pKa with solubility < 1 µM
- pKa =50% ionization ↔ (max. mobility)/2
- Mobility ≈ charge/mass

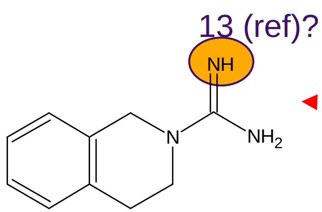
AstraZeneca

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

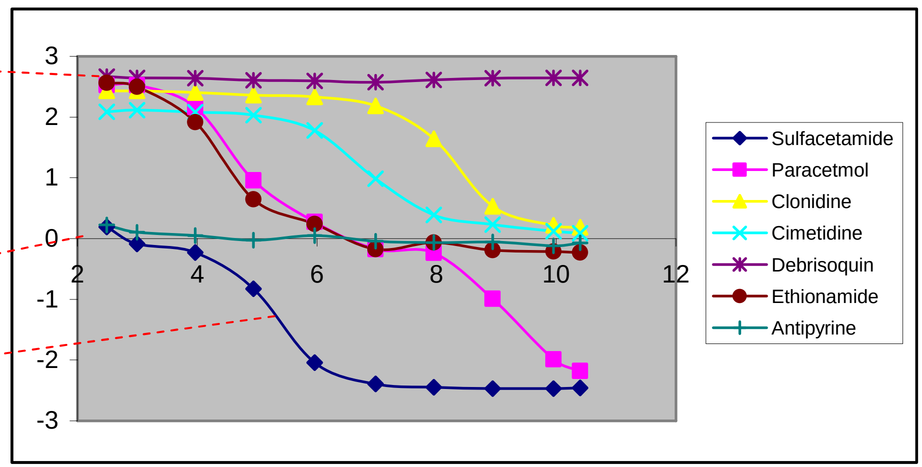


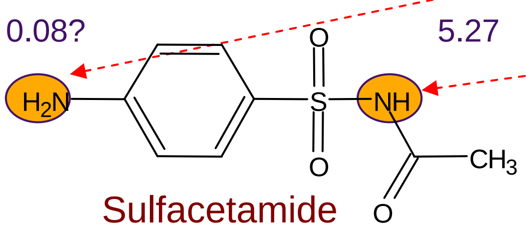
Omeprazole





Debrisoquin





Sulfacetamide

AstraZeneca

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 µM) 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

AstraZeneca