The Chromatographic Side of LC-MS and its Consequences

11/03/12

Carr's Group Meeting by Marcelo Filgueira

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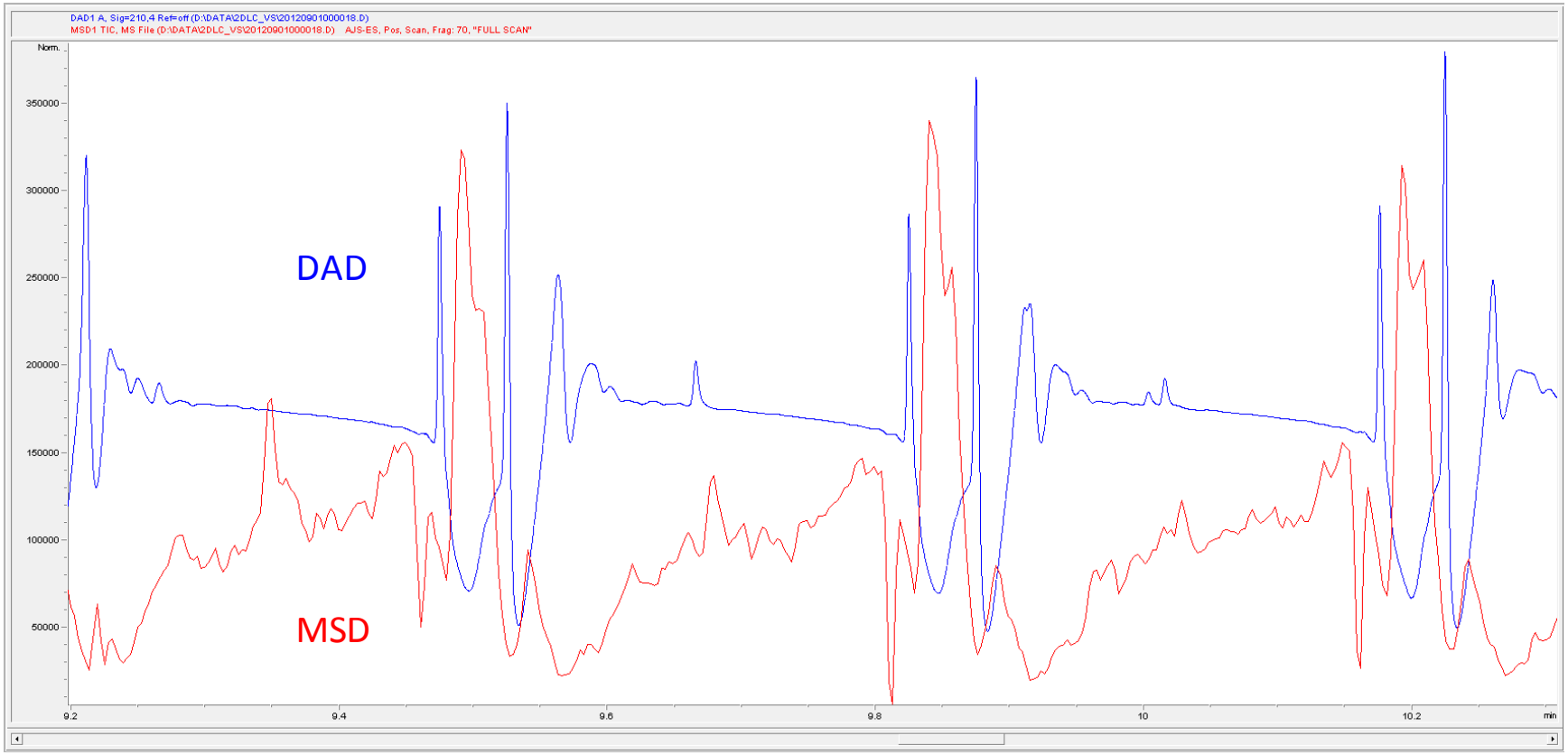
Context

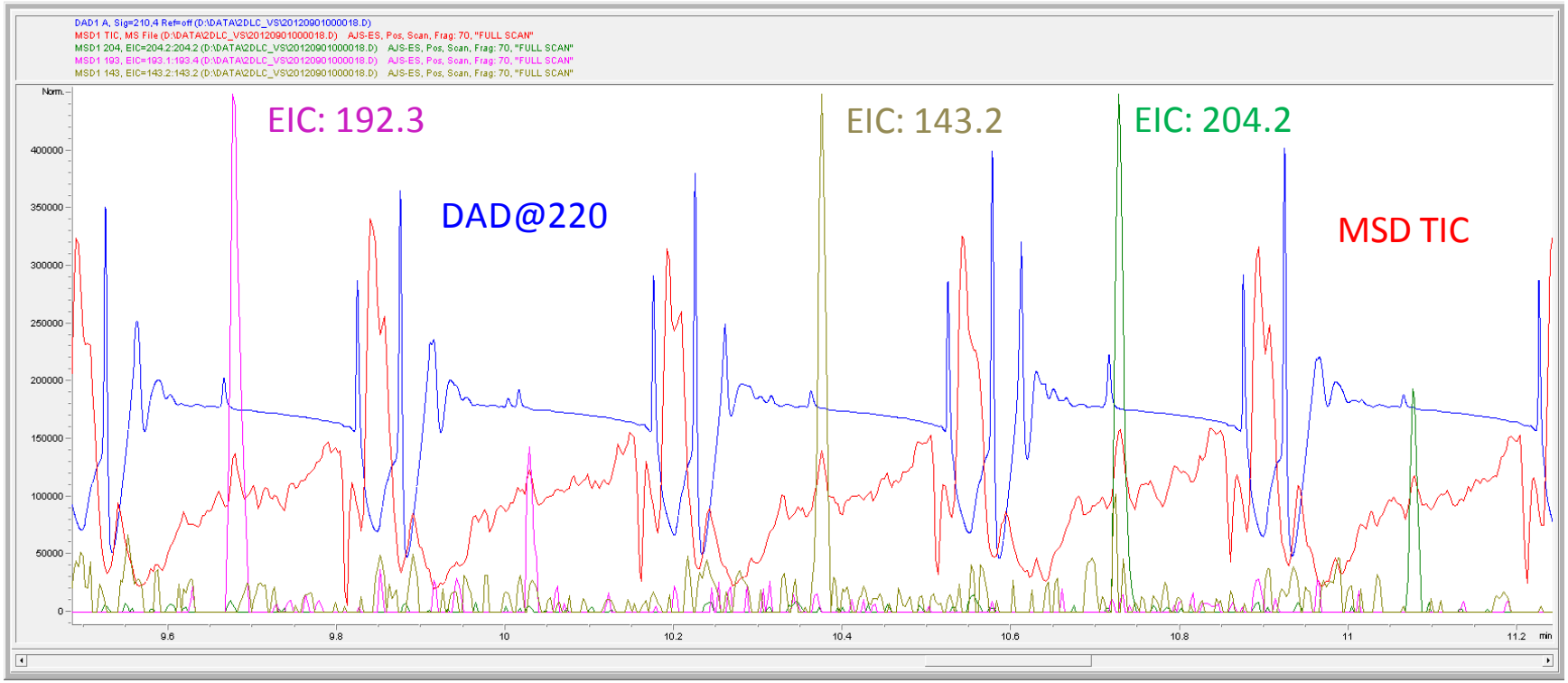
- In LC-MS you have to constrain the LC to get the benefits of MS
- When doing Fast Online LC×LC-MS we are pushing conditions to the limit
  - Very high flow rates
  - Ultra-fast gradients
  - Injections into the ^{2}D every 12-20 s
  - Scan mode for non-targeted analysis

LCxLC: DAD vs. MSD



Indoles mixture. DAD@220 nm, MSD TIC@100-700 Da, t_c: 21 s, Active Splitter: 27 µL/min
^{2}D column is SB-C18 30x2.1 3.5 um, ^2t_g: 18, ^{2}F: 1.5 mL/min

LCxLC: DAD vs. MSD



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^{2}D column is SB-C18 30x2.1 3.5 um, ^2t_g: 18, ^{2}F: 1.5 mL/min
EIC: Cyan@143.2, Pink@193.3, Green@204.2

Outline

- Background
- The Organic Modifier
- Acid Type and Concentration
- Effect of pH in Fragmentation
- Quality of the Solvents
- Matrix Effects and Ion Suppression
- Strategies To Reduce Matrix Effects

Coupling LC with MS

- Atmospheric Pressure Ionization (API)
  - Electrospray Ionization(ESI)
  - Atmospheric Pressure Chemical Ionization (APCI)
  - Atmospheric Pressure Photo-Ionization (APPI)
- ESI is currently the most widely used ionization source (80 % of publications with LC-MS)

The amount of Ions going into the source will depend on

- ES efficiency
- Protonation of molecules
- Ions observed in the ES MS do not reflect the equilibrium concentrations of ions in solution*

* Hiraoka K., Murata K., and Kudaka I., *J. Mass Spectrom. Soc. Jpn*. , **1995**, Vol. 43, No. 3

The Organic Modifier

Acetone, Methanol and Acetonitrile

Table 1. Selected physical and chemical properties of acetonitrile, methanol and acetone[1,5]

Property	Acetonitrile	Methanol	Acetone
Density [g/mL]	0.7822	0.7913	0.79
Viscosity [cP]	0.38	0.55	0.36
Boiling point [°C]	81.6	64.7	56.29
Vapor pressure [hPa at 20°C]	118.39	33.33	245.98
Elutropic strength on C18 [ε°]	3.1	1	8.8
UV cutoff [nm]	190	205	330
LD_{50} (oral; rat) [mg/kg]	2,460	5,628	5,800
LC_{50} (inhalation; rat)	7,551 ppm (8 h)	64,000 ppm (4 h)	50,100 mg/m^3 (8 h)
LD_{50} (dermal) [mg/kg]	2,000 (rabbit)	15,800 (rabbit)	7,426 (guinea pig)

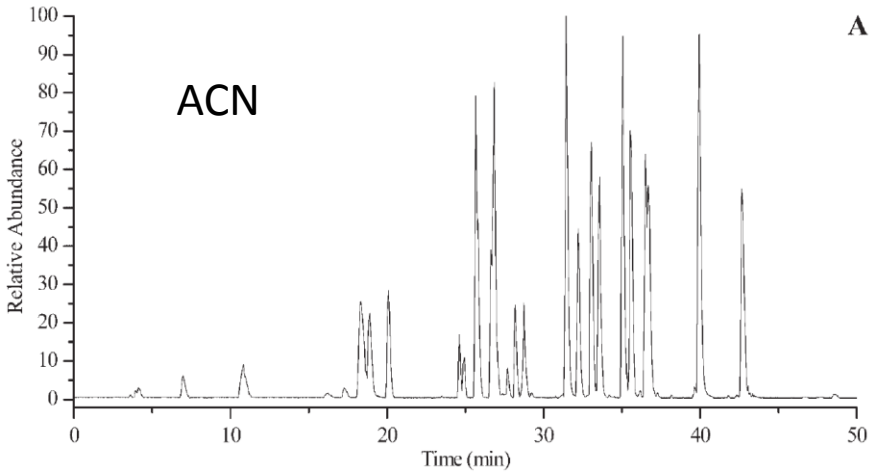
Acetonitrile with 0.1% formic acid; Methanol with 0.1% formic acid; Water with 0.1% formic acid (all LC/MS Chromatosolv) purchased from Riedel-de Haen (Seelze, Germany)

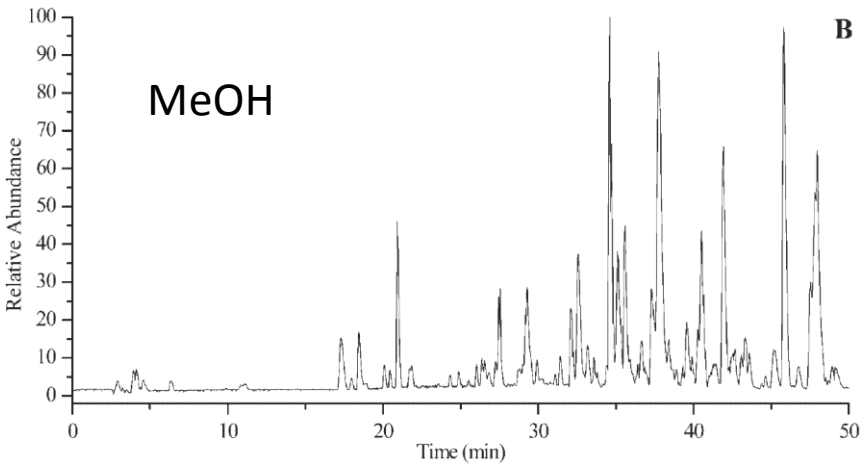
Acetone (Baker ultra resi-analysed) from J.T.Baker (Deventer, The Netherlands).

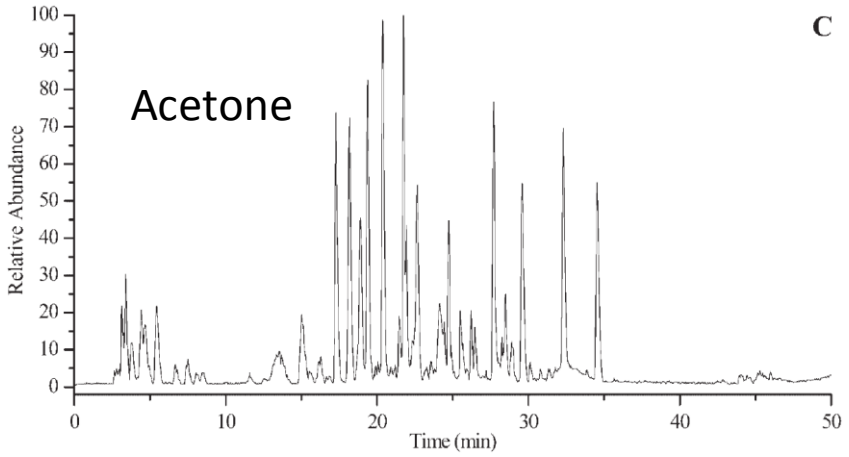
Fritz, R.; Ruth, W.; Kragl, U. *Rapid Communications in Mass Spectrometry* **2009**, *23*, 2139–2145.

Acetone, Methanol and Acetonitrile

Chromatograms of a tryptic digest of BSA using (A) ACN, (B) MeOH, and (C) Acetone







BioBasic C18, 2.1x150 mm, 5 um, 300A; Thermo Scientific

Gradient: 5% ACN in water (0.1% FA) for 10 min; linear gradient to 80% ACN in 40 min, hold for 5 min and back to 5% ACN in 5 min (Same for Methanol and Acetone)
F: 150 uL/min @ 25 C.

Fritz, R.; Ruth, W.; Kragl, U. *Rapid Communications in Mass Spectrometry* **2009**, *23*, 2139–2145.

Acetone, Methanol and Acetonitrile

Table 2. Amino acid coverage [%] of tryptic protein digests in acetonitrile, methanol and acetone

Protein	Acetonitrile	Methanol	Acetone
BSA gi|1351907	29.2	31.68	32.46
Transferrin gi|113911795	12.9	n.d.	10.89
hemoglobin, subunit alpha gi|122272	77.3	78.25	54.61
hemoglobin, subunit beta gi|122571	71.03	72.41	77.47
Myoglobin gi|2554649	66.01	23.75	69.03
Lysozyme IPI00600859.1	25.17	16.33	29.93

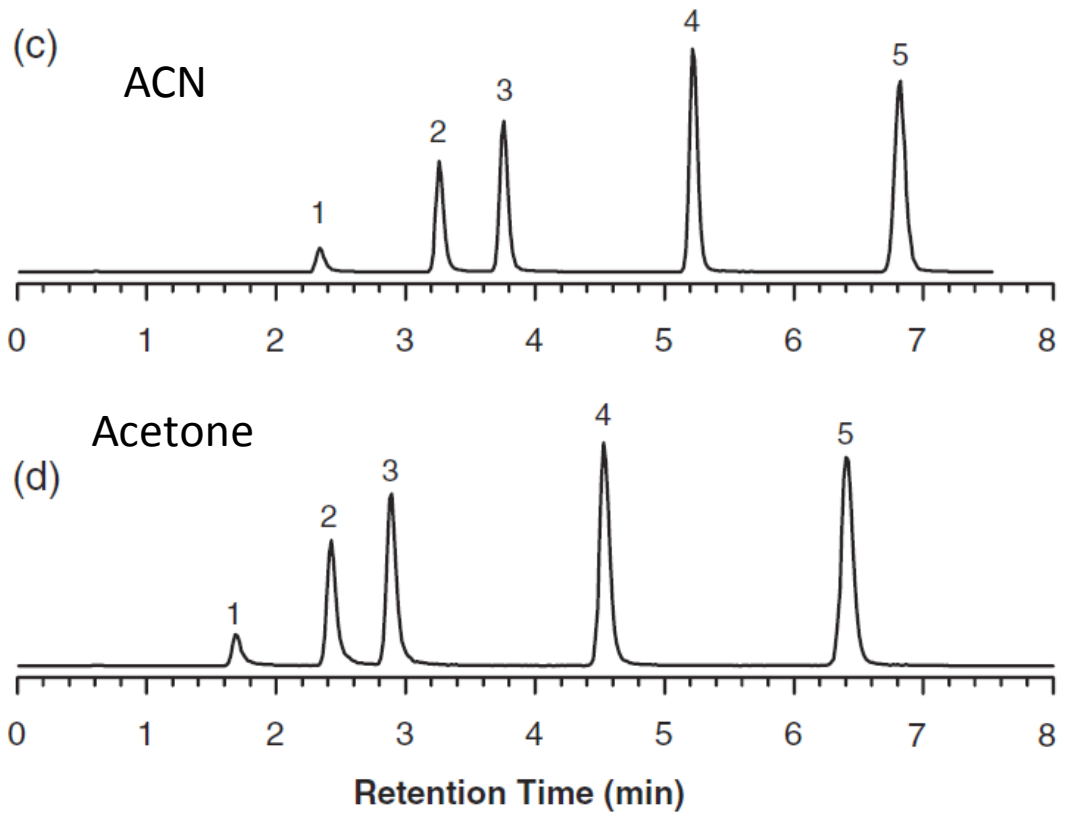
Fritz, R.; Ruth, W.; Kragl, U. *Rapid Communications in Mass Spectrometry* **2009**, *23*, 2139–2145.

Acetone and Acetonitrile

- With respect to ESI, acetone has a lower surface tension and higher volatility than acetonitrile, suggesting that ESI would be more efficient with acetone than with acetonitrile
- Finally, we note that the price of acetone is comparable to methanol and roughly one-third that of acetonitrile (Fisher Optima grade)

Keppel, T. R.; Jacques, M. E.; Weis, D. D. *Rapid Communications in Mass Spectrometry* **2010**, *24*, 6–10.

Acetone and Acetonitrile



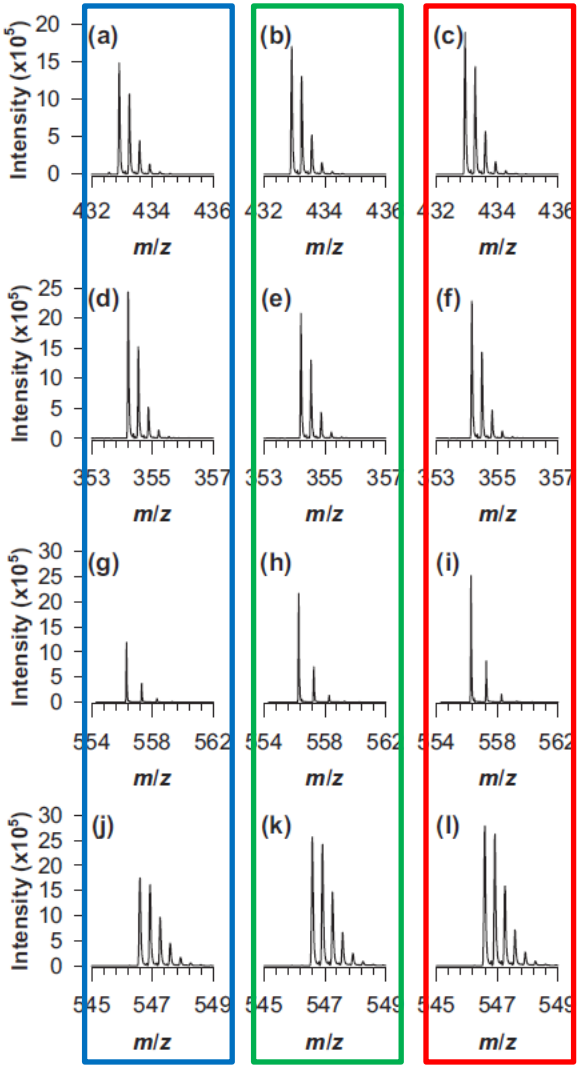
acetonitrile/water	
t_r	$w_{½}$
2.348 ± 0.012	0.115 ± 0.007
3.265 ± 0.012	0.086 ± 0.001
3.764 ± 0.010	0.082 ± 0.003
5.215 ± 0.017	0.080 ± 0.002
6.812 ± 0.023	0.104 ± 0.003

acetone/water	
t_r	$w_{½}$
1.708 ± 0.017	0.149 ± 0.007
2.434 ± 0.021	0.118 ± 0.003
2.894 ± 0.022	0.113 ± 0.002
4.550 ± 0.015	0.104 ± 0.001
6.410 ± 0.008	0.115 ± 0.001

Total ion chromatograms of the separation of the peptide retention standard. (c) C18 column with an 8–18% B acetonitrile/water gradient, and (d) C18 column with a 7–18% B acetone/water gradient. 50x1.0mm ZORBAX 300 SB-C18, 3.5 um, 300 A; F: 50 uL/min

Keppel, T. R.; Jacques, M. E.; Weis, D. D. *Rapid Communications in Mass Spectrometry* **2010**, *24*, 6–10.

Acetone and Acetonitrile



ESI+ Efficiency:
Solutions directly infused into the MSD

Maximize signal intensity of peptides dissolved in acetonitrile/water/formic acid (25/75/0.1%) directly infused into the electrospray source.

Acetone was substituted for acetonitrile as the organic solvent and spectra were acquired with TOF settings unchanged from their acetonitrile-optimized settings.

Maximized signal intensity for each peptide dissolved in acetone/water/formic acid (25/75/0.1%).

Keppel, T. R.; Jacques, M. E.; Weis, D. D. *Rapid Communications in Mass Spectrometry* **2010**, *24*, 6–10.

Acetone and Acetonitrile

ESI Efficiency: Optimized parameters

Table 2. Optimized ESI parameters for infusions of each individual peptide dissolved in either acetonitrile/water/formic acid (25/75/0.1%) or acetone/water/formic acid (25/75/0.1%)

	Capillary voltage (V)		Fragmentor voltage (V)		Gas flow (L min^{-1})		Maximum ion signal intensity ($\times 10^5$)	
Peptide	Acetonitrile	Acetone	Acetonitrile	Acetone	Acetonitrile	Acetone	Acetonitrile	Acetone
angiotensin I	3300	3500	145	160	12	12	14.9	18.9
[Leu^5]-enkephalin	3300	3800	180	190	10	10	24.4	22.8
bradykinin	3500	4000	130	110	12	12	11.9	25.2
somatostatin 14	3700	3800	150	140	12	12	17.4	27.9

Keppel, T. R.; Jacques, M. E.; Weis, D. D. *Rapid Communications in Mass Spectrometry* **2010**, *24*, 6–10.

Acetone and Acetonitrile

Comparing spectra in the m/z 50–500 region for these two solvent mixtures shows that acetone had a more complex mixture of ions at higher intensities.

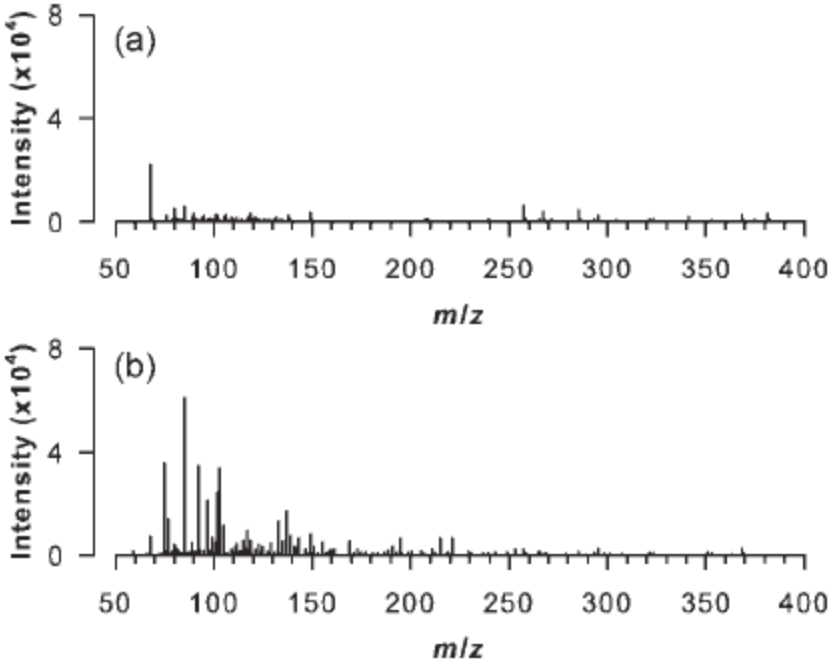


Figure 3. ESI-MS spectra from infusion of (a) acetonitrile/water/formic acid (50/50/0.1%) and (b) acetone/water/formic acid (50/50/0.1%). Centroid data with an intensity threshold of 500 are shown.

Solvents from Fisher Scientific:
Optima LC/MS grade ACN and Water
Optima grade Acetone

Keppel, T. R.; Jacques, M. E.; Weis, D. D. *Rapid Communications in Mass Spectrometry* **2010**, *24*, 6–10.

Acid Type and Concentration

Influence of Acid Type and Concentration in ESI+ (Proteins)

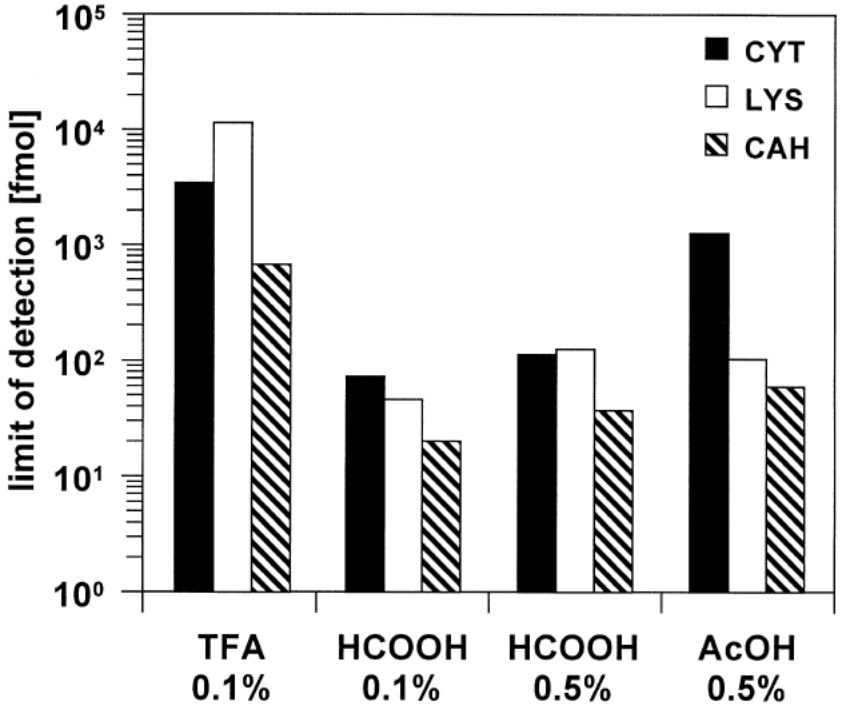


Fig. 4. Influence of mobile phase additive on limits of detection of proteins. Column, PS–DVB–C_{18} (2.3 μm, 60×1.0 mm I.D.); linear gradient, 19.5–60% acetonitrile in 0.10% aqueous trifluoroacetic acid, 0.10% aqueous formic acid, 0.50% aqueous formic acid, and 0.50% aqueous acetic acid, respectively, in 10 min; flow-rate, 30 μl/min; temperature, 80°C; detection, ESI-MS, electrospray voltage, 4.5 kV; scan range, 500–2500 u in 2.0 s; sample, CYT, LYS, CAH.

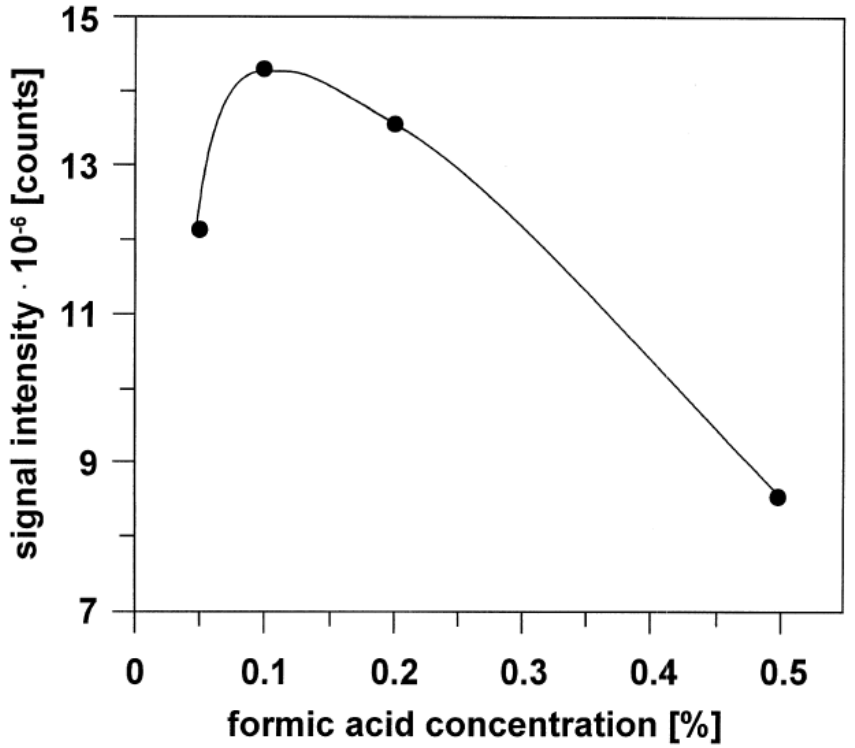


Fig. 2. Influence of formic acid concentration on ESI-MS signal intensity. Direct infusion of 0.10 mg/ml lysozyme in acetonitrile–water (50:50) containing 0.050–0.50% formic acid. Flow-rate, 3.0 μl/min; scan, 1000–2400 u in 1.0 s.

Huber, C. G.; Premstaller, A. *Journal of Chromatography A* **1999**, *849*, 161–173.

Influence of Acid Type and Concentration in ESI+ (Proteins)

Peak widths at half height w_h observed with trifluoroacetic acid, formic acid and acetic acid as mobile phase additives using UV and full-scan ESI-MS detection

Additive	w_h[a] (s)					
	UV[b]			MS[c]		
	CYT	LYS	CAH	CYT	LYS	CAH
Trifluoroacetic acid, 0.1%	7.6	7.7	8.0	8.0	8.0	12.0
Formic acid, 0.1%	19.3	16.5	21.9	26.2	21.2	14.0
Formic acid, 0.5%	15.5	13.0	14.5	18.5	17.5	15.5
Acetic acid, 0.5%	27.6	29.4	24.0	30.0	36.7	32.5

[a] Gradient, 19.5–60% acetonitrile in 10 min, 30 µl/min, 80°C.
[b] Wavelength, 215 nm.
[c] Scan, 500–2500 u in 2 s.

Standard proteins used in this study

Protein	Abbreviation	Source
Carbonic anhydrase	CAH	Bovine erythrocytes
Cytochrome *c*	CYT	Horse heart
α-Lactalbumin	LALB	Bovine milk
β-Lactoglobulin A	LAC A	Bovine milk
β-Lactoglobulin B	LAC B	Bovine milk
Lysozyme	LYS	Chicken egg white
Myoglobin	MYO	Horse heart
Ribonuclease A	RIB	Bovine pancreas
Transferrin	TRA	Human
Trypsin	TRY	Bovine pancreas

Huber, C. G.; Premstaller, A. *Journal of Chromatography A* **1999**, *849*, 161–173.

Effect of Weak Acids on ESI-

"…different acids had quite different effects on the negative-ion ESI responses of these compounds, indicating that the properties of the acid played an important role in analyte deprotonation."

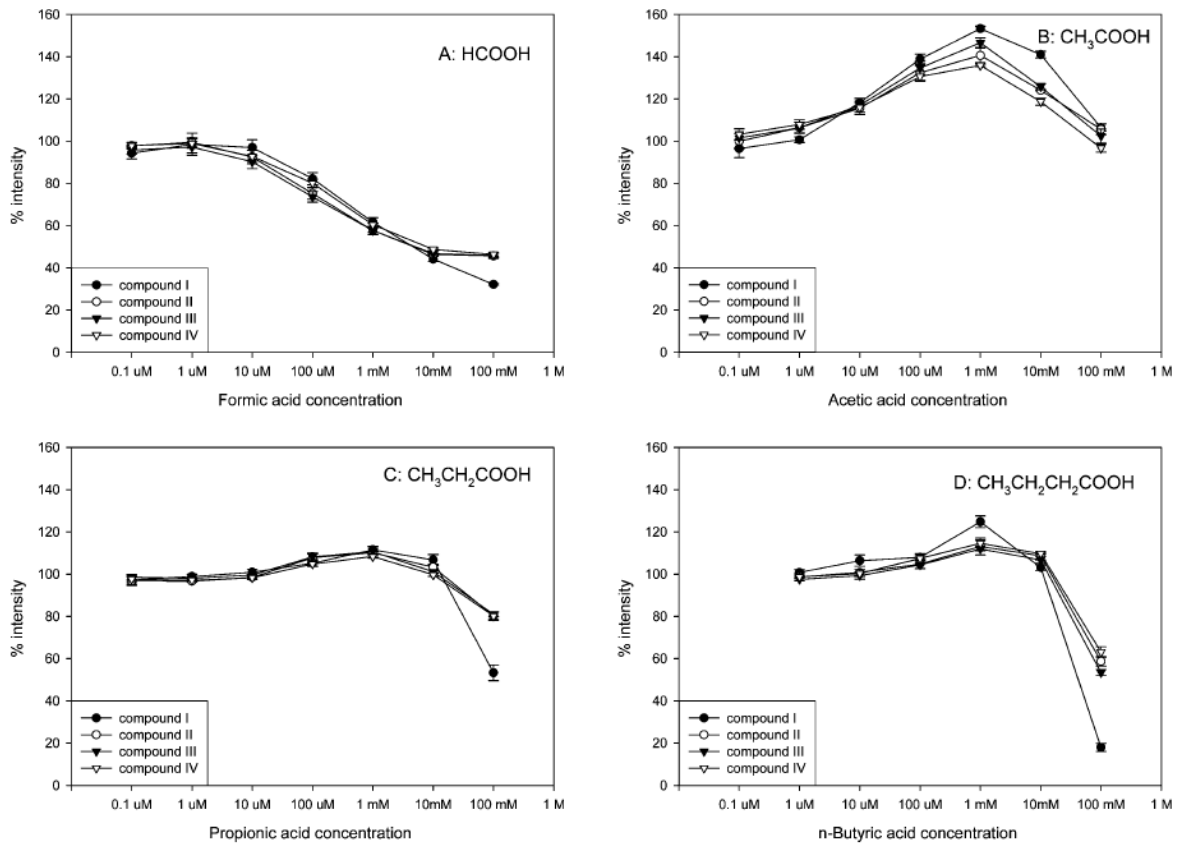


Figure 3. Effects of carboxylic acids on the negative-ion ESI responses of four SARMs. The horizontal axis represents the final concentration of modifier in the flow before entering the ESI source. The vertical axis represents the mean (±SD, $N = 3$) ratio of (peak area of each compound in the presence of modifier) to (the peak area of each compound in the absence of modifier), multiplied by 100%.

Wu, Z.; Gao, W.; Phelps, M. A.; Wu, D.; Miller, D. D.; Dalton, J. T. *Analytical Chemistry* **2004**, *76*, 839–847.

Effect of Weak Bases on ESI-

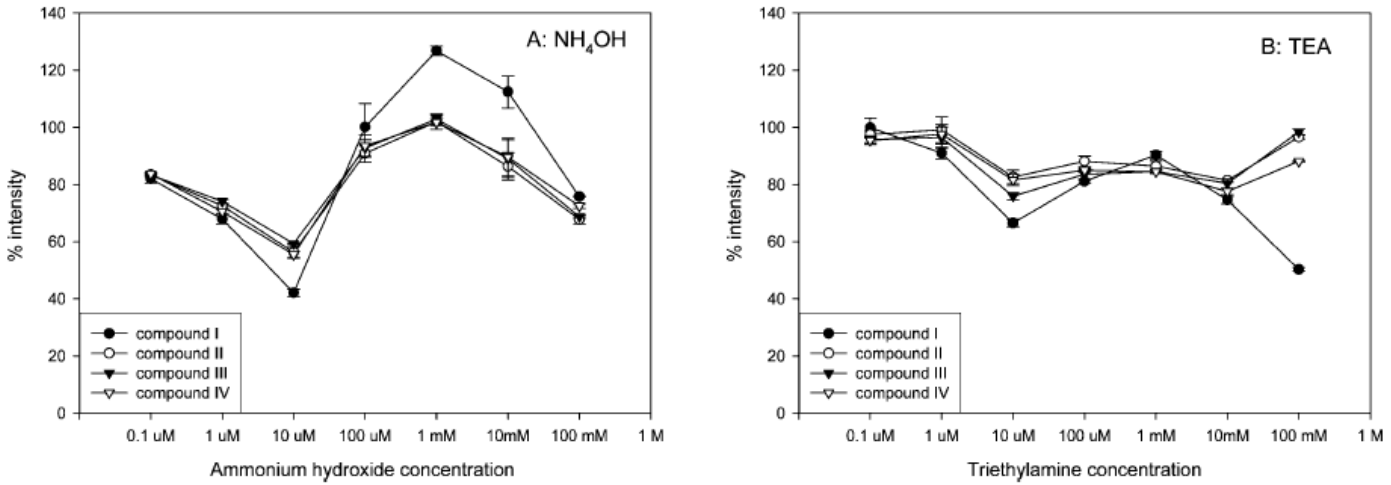


Figure 4. Effect of volatile bases on negative-ion ESI responses of four SARMs. The horizontal axis represents the final concentration of modifier in the flow before entering the ESI source. The vertical axis represents the mean (±SD, $N = 3$) ratio of (peak area of each compound in the presence of modifier) to (the peak area of each compound in the absence of modifier), multiplied by 100%.

Table 1. pK_a Values of Some Reagents Used in the Present Study and Their Gas-Phase Gibbs Free Energies of Formation

compounds	pK_a [27]	reaction	$\Delta_r G°$ (kJ/mol)
formic acid	3.75	$HCOO^- + H^+ = HCOOH$	1415.0 ± 8.4 [28]
acetic acid	4.746	$CH_3COO^- + H^+ = CH_3COOH$	1427.0 ± 8.4 [29]
propionic acid	4.87	$CH_3CH_2COO^- + H^+ = CH_3CH_2COOH$	1424.0 ± 8.4 [30]
n-butyric acid	4.83	$CH_3CH_2CH_2COO^- + H^+ = CH_3CH_2CH_2COOH$	1420.0 ± 8.4 [28]
2,2,2-trifluoroethanol	12.37	$CF_3CH_2O^- + H^+ = CF_3CH_2OH$	1482.0 ± 8.4 [30]
formaldehyde	13.27	$CHO^- + H^+ = HCOH$	1618.0 ± 1.3 [31]
water	13.995	$HO^- + H^+ = H_2O$	1605.4 ± 1.3 [32]

Wu, Z.; Gao, W.; Phelps, M. A.; Wu, D.; Miller, D. D.; Dalton, J. T. *Analytical Chemistry* **2004**, *76*, 839–847.

Effect of pH on MS/MS Fragmentation

…"These charge-site isomers have identical m/z values but different sites of proton attachment.

The different charge-site isomers can fragment independently and the resulting MS/MS spectrum in a given mobile phase is a composite of these product ion spectra and reflective of the mixture of ions present in the gas phase when electrosprayed from solution of a specific composition."

Wang, J.; Aubry, A.; Bolgar, M. S.; Gu, H.; Olah, T. V.; Arnold, M.; Jemal, M. *Rapid Communications in Mass Spectrometry* **2010**, *24*, 3221–3229.

Effect of pH on MS/MS Fragmentation

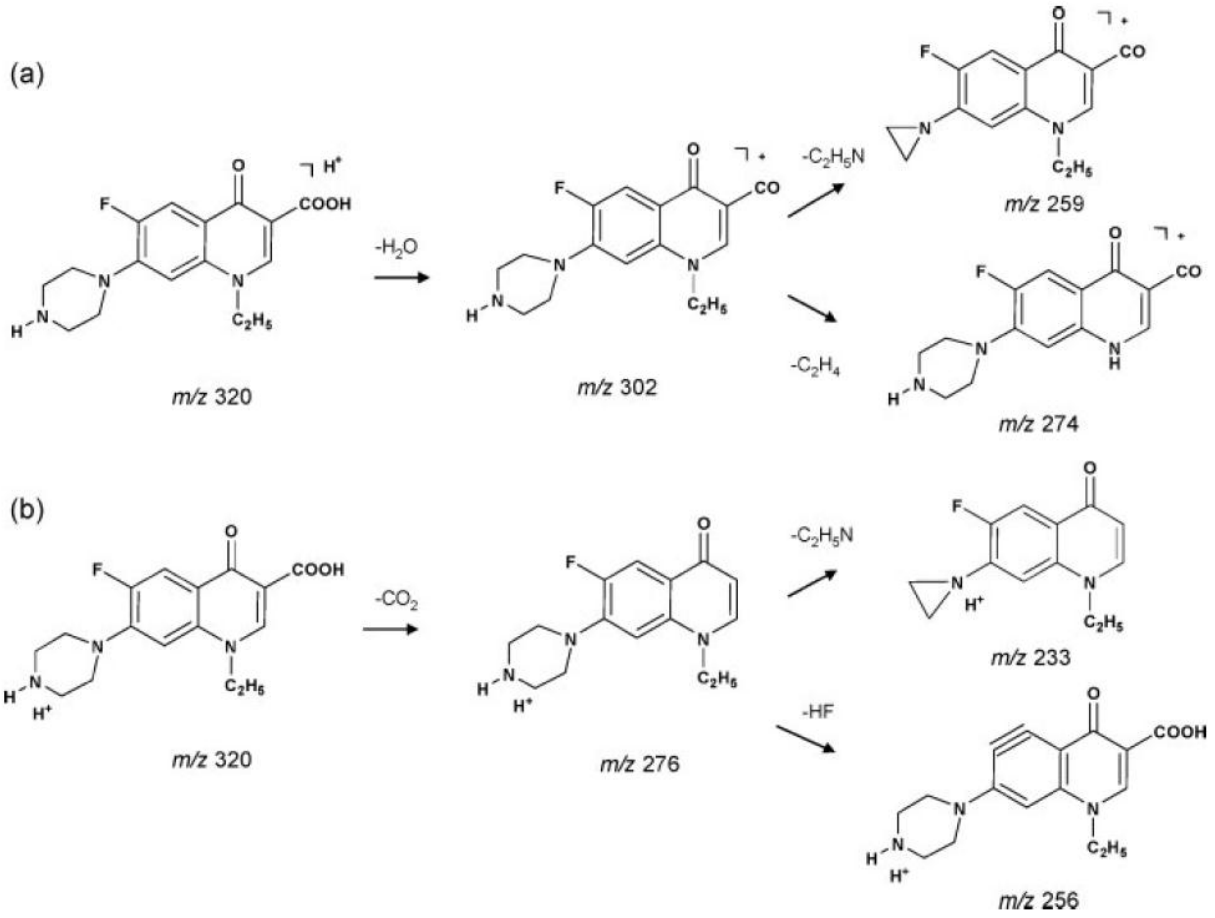


Figure 5. MS/MS fragmentation pathways of norfloxacin $[M+H]^+$.

Wang, J.; Aubry, A.; Bolgar, M. S.; Gu, H.; Olah, T. V.; Arnold, M.; Jemal, M. *Rapid Communications in Mass Spectrometry* **2010**, *24*, 3221–3229.

Effect of pH on MS/MS Fragmentation

Table 5. Summary of the effect of various mobile phase parameters on MS/MS fragmentation patterns of three pharmaceutical compounds

Compound / Effect	Difloxacin	Marbofloxacin	Norfloxacin
pH (pH 3.3 vs. 9.3)	Yes	Yes	Minor*
Aqueous-organic ratio			
pH 3.3	Minor**	Minor**	Minor**
pH 9.3	No	No	Yes***
Buffer concentration			
pH 3.3	Yes	Yes	Yes
pH 9.3	No	No	No

* Different fragmentation pattern at pH 3.3 vs. 9.3 observed at 95% acetonitrile only.

** Small continuous change of fragmentation pattern when aqueous-organic ratio changed.

*** Significantly different fragmentation pattern between 95% acetonitrile and 75% acetonitrile. Minor effect at lower % of acetonitrile.

Wang, J.; Aubry, A.; Bolgar, M. S.; Gu, H.; Olah, T. V.; Arnold, M.; Jemal, M. *Rapid Communications in Mass Spectrometry* **2010**, *24*, 3221–3229.

Quality of the Mobile Phase

- The quality and suitability of all the mobile phase constituents must be considered because they all enter the ion source and influence ion generation. (1)

- In evaluating the response of 32-desmethoxyrapamycin using 9 brands of methanol from 5 different manufacturers, they demonstrated that the MS/MS peak area could vary by at least an order of magnitude (10-fold).(2)

(1) Gray, M. J.; Jahani, S.; Low, G. K.-C. *Journal of Chromatography A* **2012**, *1219*, 83–92.

(2) T.M. Annesley, Clin. Chem. 53 (2007) 1827.

Quality of the Mobile Phase

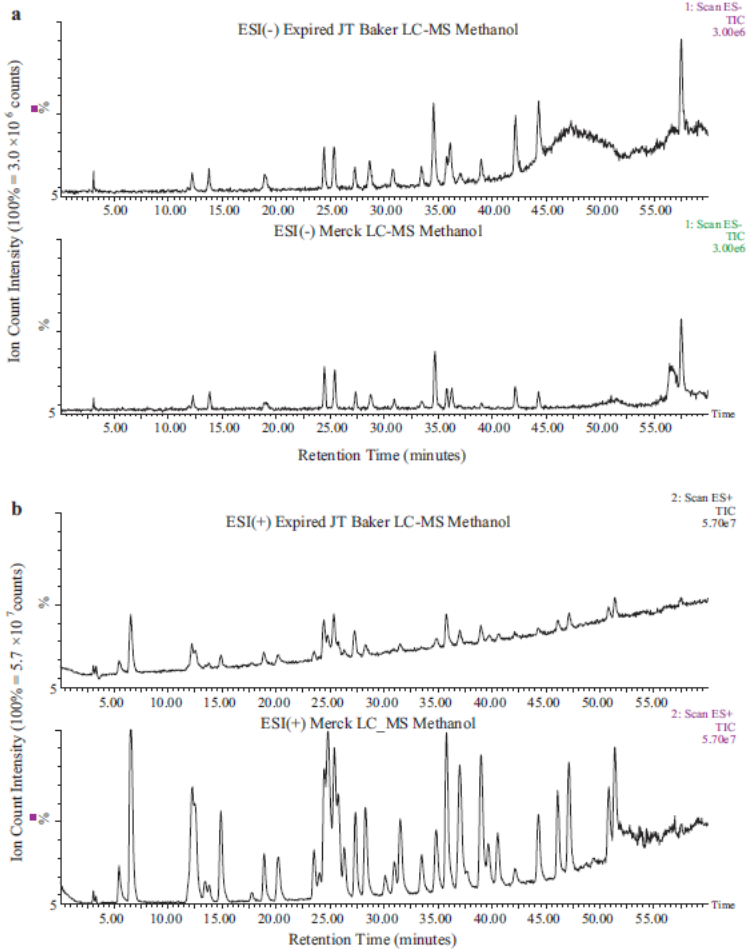


Fig. 1. Total ion chromatograms of a mixture of herbicides, fungicides and insecticides using two different brands of methanol using (a) ESI(−) mode and (b) ESI(+) mode.

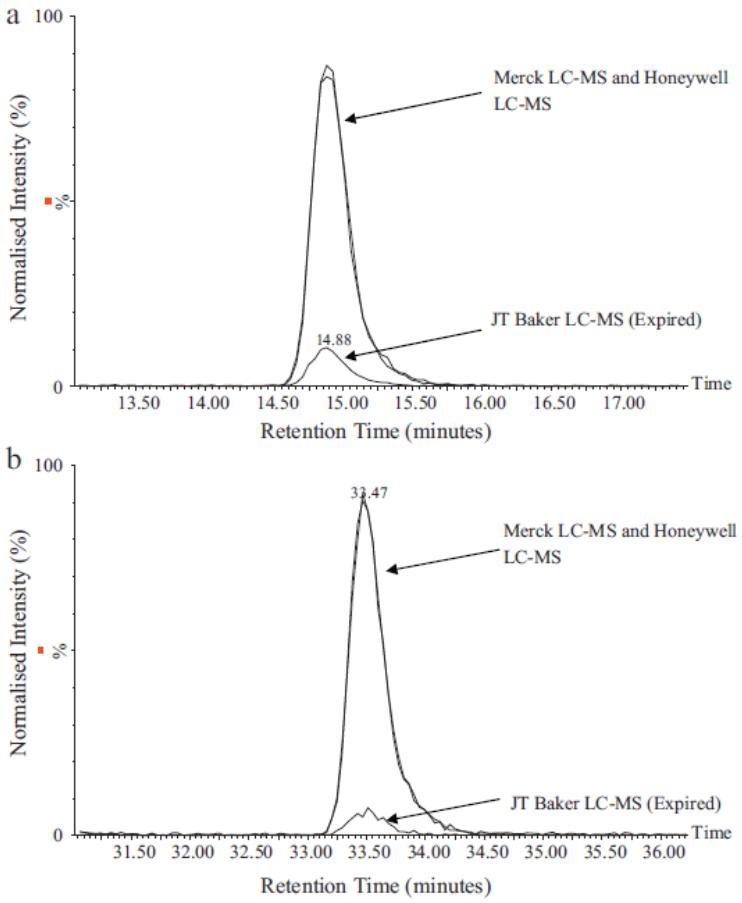


Fig. 2. ESI(+) extracted ion chromatograms of (a) dimethoate at $m/z = 199$ and (b) diuron at $m/z = 233$ using different brands of methanol as a mobile phase component.

Gray, M. J.; Jahani, S.; Low, G. K.-C. *Journal of Chromatography A* **2012**, *1219*, 83–92.

Quality of the Mobile Phase

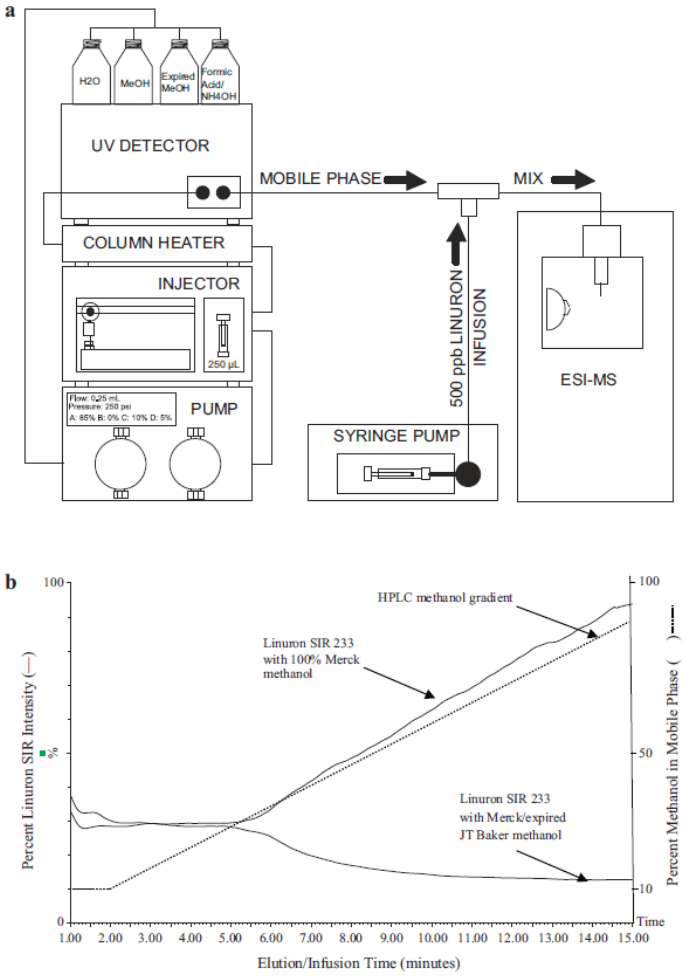


Fig. 3. (a) Schematics for the linuron infusion experiment. (b) ESI(+) SIR infusion profiles at m/z = 233 of a 500 ppb linuron solution when using methanol gradient elution.

Gray, M. J.; Jahani, S.; Low, G. K.-C. *Journal of Chromatography A* **2012**, *1219*, 83–92.

Quality of the Mobile Phase

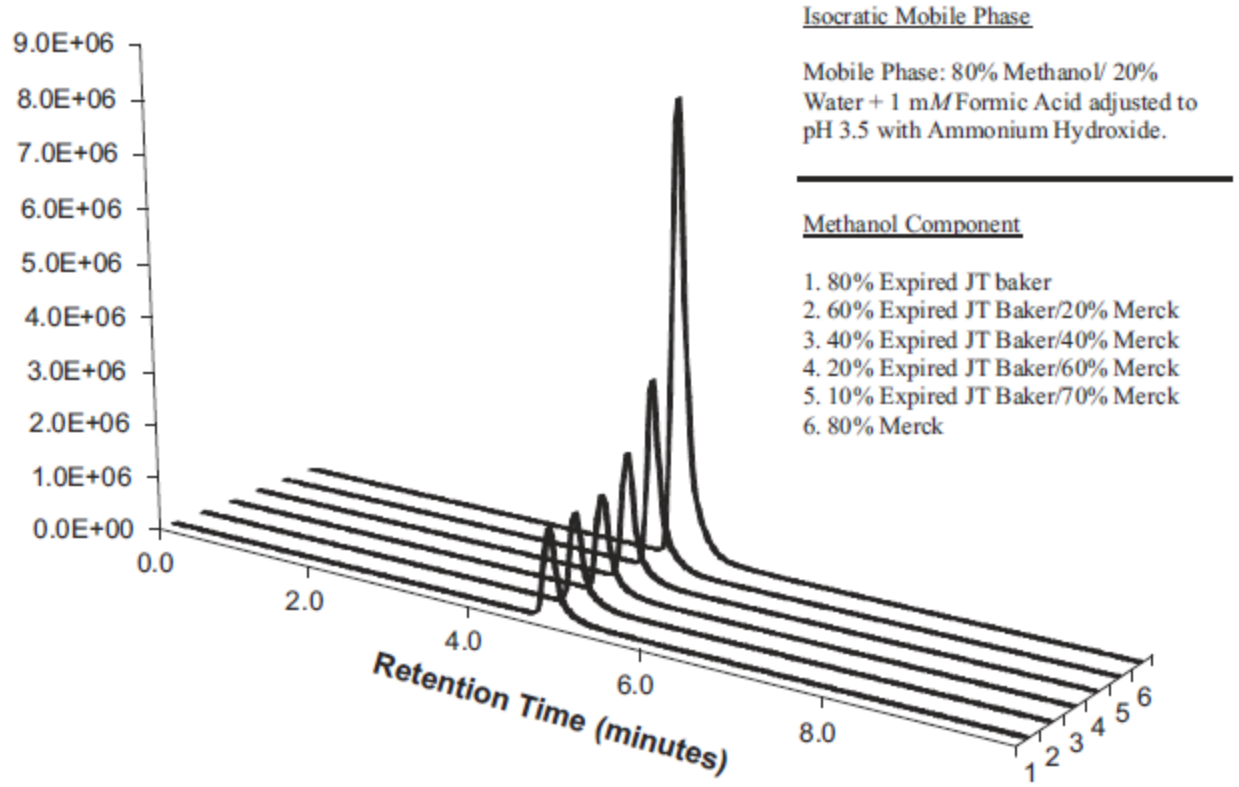


Fig. 4. The changing ESI(+) signal response of linuron with a change in the ratio of expired JT Baker LC–MS Methanol to Merck LC–MS Methanol.

Gray, M. J.; Jahani, S.; Low, G. K.-C. *Journal of Chromatography A* **2012**, *1219*, 83–92.

Matrix Effects

- Matrix effects are the alteration of ionization efficiency by the presence of co-eluting substances
- The exact mechanism of matrix effects is unknown
  - Competition between nonvolatile matrix components and analyte ions for access to the droplet surface for transfer to the gas phase
- The majority of matrix effects occur in the solvent front of a chromatographic run (careful….)

Matrix Effects

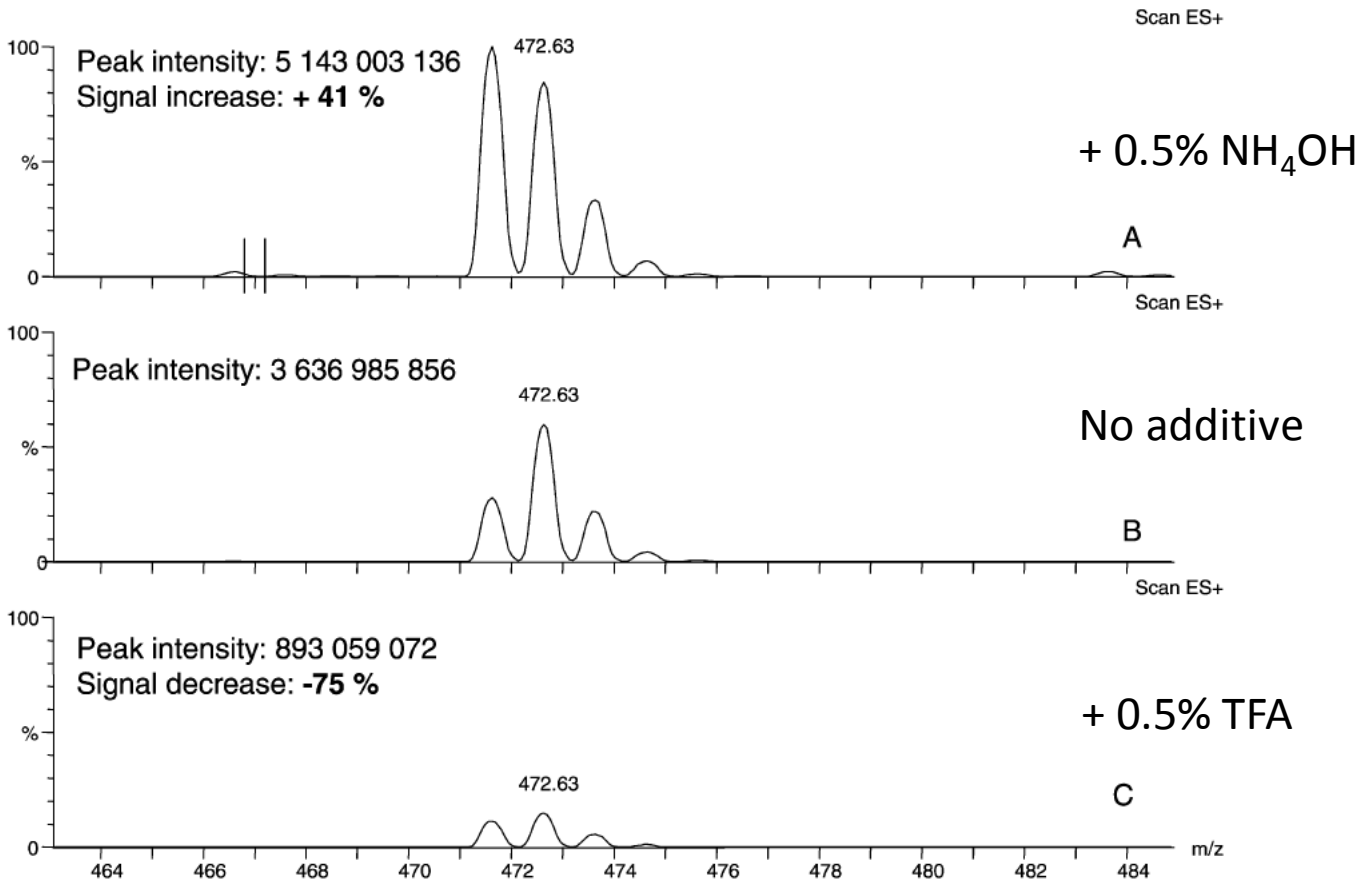


Figure 2. Examples of ion suppression and enhancement on terfenadine. (A) Terfenadine mixed with 50/50 MeOH/H_2O + 0.5% NH_4OH; (B) terfenadine mixed with 50/50 MeOH/H_2O + no additive; and (C) terfenadine mixed with 50/50 MeOH/H_2O + 0.5% TFA.

Mallet, C. R.; Lu, Z.; Mazzeo, J. R. *Rapid Communications in Mass Spectrometry* **2004**, *18*, 49–58.

Matrix Effects: pH Additives

Table 3. Suppression and enhancement effects for the pH additives

	0.05%	0.10%	0.50%	1.00%	0.05%	0.10%	0.50%	1.00%
	Formic acid				Ammonium hydroxide			
Positive test solution								
Propranolol	36.5	28.8	4.5	−8.3	−2.2	2.02	10.2	11.4
Trimethoprim	41.7	30.1	−5.3	−17.5	−5.4	−5.4	4.2	8.9
Pipenzolate	−0.1	−0.2	−5.5	−9.5	0.02	0.02	0.02	0.01
Resperidone	−27.5	−37.1	−54.2	−59.4	6.1	9.6	16.1	16.8
Terfenadine	17.3	11.6	−7.9	−16.5	10.8	21.3	57.9	66.6
Methoxyverapamil	22.8	17.1	−1.8	−10.7	38.8	41.1	46.6	49.1
Benextramine	−39.77	−44.1	−52.7	−52.8	22.1	30.7	37.9	38.3
Reserpine	21.4	21.4	17.2	8.9	−12.1	−11.9	−6.2	−3.2
Negative test solution								
Fumaric acid	−11.9	−29.5	−64.7	−68.1	−38.4	−41.1	−45.8	−57.8
Malic acid	−11.2	−27.9	−62.2	−63.9	−35.5	−38.8	−42.4	−53.4
Etidronic acid	29.8	17.8	−17.2	−30.9	−61.9	−63.5	−75.9	−70.3
Clodronic acid	5.7	−15.7	−58.3	−66.6	0.3	−1.3	−5.3	−27.7
Niflumic acid	−0.28	−21.4	−60.9	−64.5	14.1	11.1	5.3	−11.6
Canrenoic acid	13.8	−11.1	−51.6	−57.6	196.1	202.5	201.9	127.3
Cholic acid	31.9	3.7	−40.8	−44.7	420.5	454.9	403.1	352.8
Raffinose	−4.6	−26.3	−39.4	−43.7	60.9	61.9	66.6	32.1
	Trifluoroacetic acid				Acetic acid			
Positive test solution								
Propranolol	−54.8	−62.8	−74.7	−77.1	25.5	25.8	17.3	−0.2
Trimethoprim	−40.1	−58.1	−73.9	−76.6	18.3	10.4	−0.4	−7.1
Pipenzolate	−27.5	−37.4	−43.9	−43.7	0.01	−0.01	−0.4	−1.7
Resperidone	−53.7	−62.3	−68.2	−69.3	−2.1	−16.8	−37.7	−44.2
Terfenadine	−24.4	−44.6	−61.5	−64.8	15.9	11.9	7.5	−2.8
Methoxyverapamil	−59.9	−57.3	−70.2	−72.6	19.5	16.6	8.9	−4.8
Benextramine	−29.4	−41.8	−42.7	−38.7	−21.9	−28.9	−29.9	−27.8
Reserpine	−32.5	−52.8	−71.7	−75.7	19.3	15.6	12.4	11.1
Negative test solution								
Fumaric acid	−87.4	−89.7	−91.1	−91.2	−15.1	−29.1	−51.3	−59.5
Malic acid	−84.1	−86.9	−88.4	−88.1	−14.5	−27.6	−48.3	−58.04
Etidronic acid	−71.9	−73.1	−71.6	−65.9	29.2	26.8	10.1	−17.8
Clodronic acid	−95.6	−97.4	−98.8	−98.8	4.9	−4.9	−36.5	−49.6
Niflumic acid	−91.7	−94.8	−98.2	−98.1	38.5	20.6	−22.1	−34.7
Canrenoic acid	−93.8	−96.1	−96.8	−96.1	−16.7	−33.8	−67.4	−59.4
Cholic acid	−95.2	−97.5	−99.5	−99.6	−18.9	−33.1	−48.5	−63.9
Raffinose	−84.1	−91.2	−96.5	−97.6	−5.7	−19.4	−26.3	−63.6

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Matrix Effects: Buffers

Table 4. Suppression and enhancement effects for the buffer additives

	5 mM	10 mM	20 mM	50 mM	5 mM	10 mM	20 mM	50 mM
	Ammonium formate				Ammonium biphosphate			
Positive test solution								
Propranolol	−37.5	−46.9	−56.5	−61.1	−20.2	−27.7	−28.6	−29.6
Trimethoprim	−15.8	−26.2	−36.9	−41.4	3.5	−11.6	−24.4	−37.1
Pipenzolate	−5.3	−9.1	−12.4	−14.3	−9.3	−12.3	−14.8	−15.4
Resperidone	−22.4	−25.4	−29.1	−29.6	−17.1	−29.8	−38.9	−47.7
Terfenadine	−18.2	−24.9	−29.4	−31.3	40.3	38.5	27.2	4.4
Methoxyverapamil	−30.7	−36.9	−42.4	−44.3	−7.8	−7.3	−5.5	−4.7
Benextramine	−2.1	−5.9	−9.7	−15.5	−56.5	−65.7	−69.7	−79.3
Reserpine	−8.9	−19.3	−31.9	−42.3	22.2	11.9	2.1	−5.8
Negative test solution								
Fumaric acid	−73.7	−78.8	−84.2	−86.7	−59.8	−76.5	−84.7	−90.4
Malic acid	−67.6	−72.5	−79.3	−85.1	−61.3	−76.7	−83.1	−86.5
Etidronic acid	−75.7	−80.3	−85.7	−90.5	−44.3	−73.3	−69.1	−73.7
Clodronic acid	−58.5	−65.8	−73.5	−78.7	−47.3	−72.3	−81.7	−88.5
Niflumic acid	−35.2	−41.2	−48.6	−52.4	−52.9	−75.8	−83.2	−92.9
Canrenoic acid	21.7	3.8	−12.7	−24.9	−54.7	−75.3	−85.6	−93.8
Cholic acid	93.1	68.7	45.8	23.2	−72.2	−89.6	−91.9	−97.3
Raffinose	−3.7	−8.8	−23.1	−35.8	−89.5	−95.9	−96.9	−97.3
	Ammonium bicarbonate				Nonafluoropentadecanoic acid			
Positive test solution								
Propranolol	−28.3	−40.4	−49.7	−55.6	−63.2	−68.2	−67.8	−62.1
Trimethoprim	−0.4	−15.3	−26.7	−32.6	−37.5	−50.5	−60.7	−58.8
Pipenzolate	−4.4	−9.8	−11.7	−12.7	−15.1	−32.9	−49.3	−47.1
Resperidone	−5.8	−6.8	−5.5	−2.9	−58.9	−67.1	−70.7	−64.3
Terfenadine	−1.7	−9.5	−12.7	−13.4	−46.1	−52.2	−59.7	−51.2
Methoxyverapamil	−16.3	−21.8	−25.3	−26.3	−46.8	−58.3	−64.7	−60.8
Benextramine	−19.4	−30.8	−36.5	−39.3	−15.9	−55.7	−33.8	−10.1
Reserpine	3.8	−7.3	−19.2	−28.8	−55.6	−76.5	−76.3	−77.7
Negative test solution								
Fumaric acid	−64.7	−68.9	−76.2	−78.1	−97.6	−98.5	−99.3	−99.5
Malic acid	−61.5	−65.9	−74.2	−76.3	−98.4	−98.4	−98.4	−98.7
Etidronic acid	−68.3	−72.2	−78.1	−80.8	−95.2	−96.4	−92.3	−89.9
Clodronic acid	−48.8	−54.1	−63.6	−66.3	−97.8	−98.4	−98.7	−98.6
Niflumic acid	−30.5	−35.3	−39.2	−41.7	−97.4	−97.9	−97.9	−98.1
Canrenoic acid	38.8	22.1	−7.8	−21.2	−97.2	−97.3	−97.2	−98.4
Cholic acid	233.4	196.9	104.4	83.4	−96.1	−96.7	−98.4	−99.1
Raffinose	4.6	−7.5	−25.4	−34.1	−91.8	−92.1	−96.8	−97.2

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Matrix Effects: Sample Clean-up

Table 5. Suppression and enhancement effects vs. SPE extract of plasma. A: protein precipitation; B: 1D reversed phase; C: 2D reversed phase; and D: mixed mode

	Rat plasma				Human plasma			
	A	B	C	D	A	B	C	D
Positive test solution								
Propranolol	−98.3	−42.2	−55.5	−9.1	−97.6	−28.8	−63.5	0.8
Trimethoprim	−98.2	−26.9	−55.2	−11.9	−96.6	−12.4	−61.5	−0.9
Pipenzolate	−87.6	−9.5	−39.6	−0.5	−86.4	−13.1	−48.3	2.3
Resperidone	−94.6	−32.2	−66.2	−13.5	−93.5	−22.8	−67.8	−6.4
Terfenadine	−92.9	−23.2	−39.5	−9.3	−93.5	−11.7	−41.3	0.2
Methoxyverapamil	−95.9	−38.6	−49.6	−2.6	−95.1	−28.1	−53.8	5.7
Benextramine	−42.9	26.2	−20.1	−8.2	−89.1	30.1	−6.8	−4.3
Reserpine	−94.9	−49.5	−39.9	−8.8	−93.1	−28.2	−43.9	−1.9
Negative test solution								
Fumaric acid	−99.3	na	−18.4	−11.1	−99.4	na	−88.8	−16.7
Malic acid	−98.9	na	−17.2	−10.9	−99.1	na	−86.3	−16.1
Etidronic acid	−99.2	na	−25.4	−27.5	−99.2	na	−90.9	−37.1
Clodronic acid	−95.9	na	−20.6	−12.5	−95.9	na	−79.3	−7.5
Niflumic acid	−82.2	na	−16.8	−0.4	−75.2	na	−57.6	4.6
Canrenoic acid	−88.3	na	−8.7	22.5	−86.1	na	−36.9	30.7
Cholic acid	−87.6	na	−12.1	8.5	−82.6	na	−21.4	20.1
Raffinose	−65.6	na	−44.6	4.6	−65.6	na	−50.4	−0.5

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Matrix Effects: Sample Clean-up

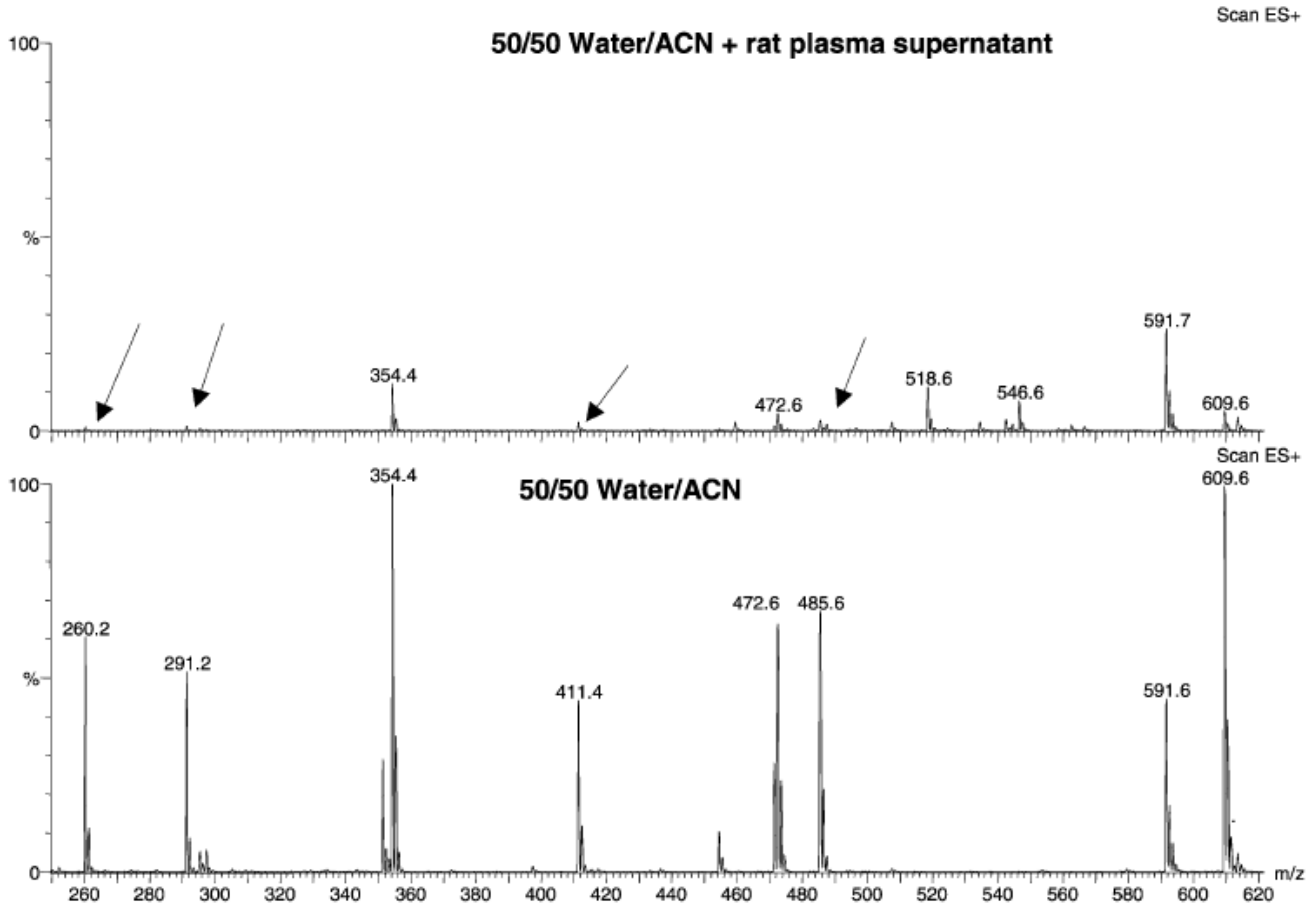


Figure 3. Mass spectra of solution of basic test analytes infused with rat plasma supernatant extract compared with result when infused with pure solvent (same scale).

Mallet, C. R.; Lu, Z.; Mazzeo, J. R. *Rapid Communications in Mass Spectrometry* **2004**, *18*, 49–58.

Matrix Effects: Sample Clean-up

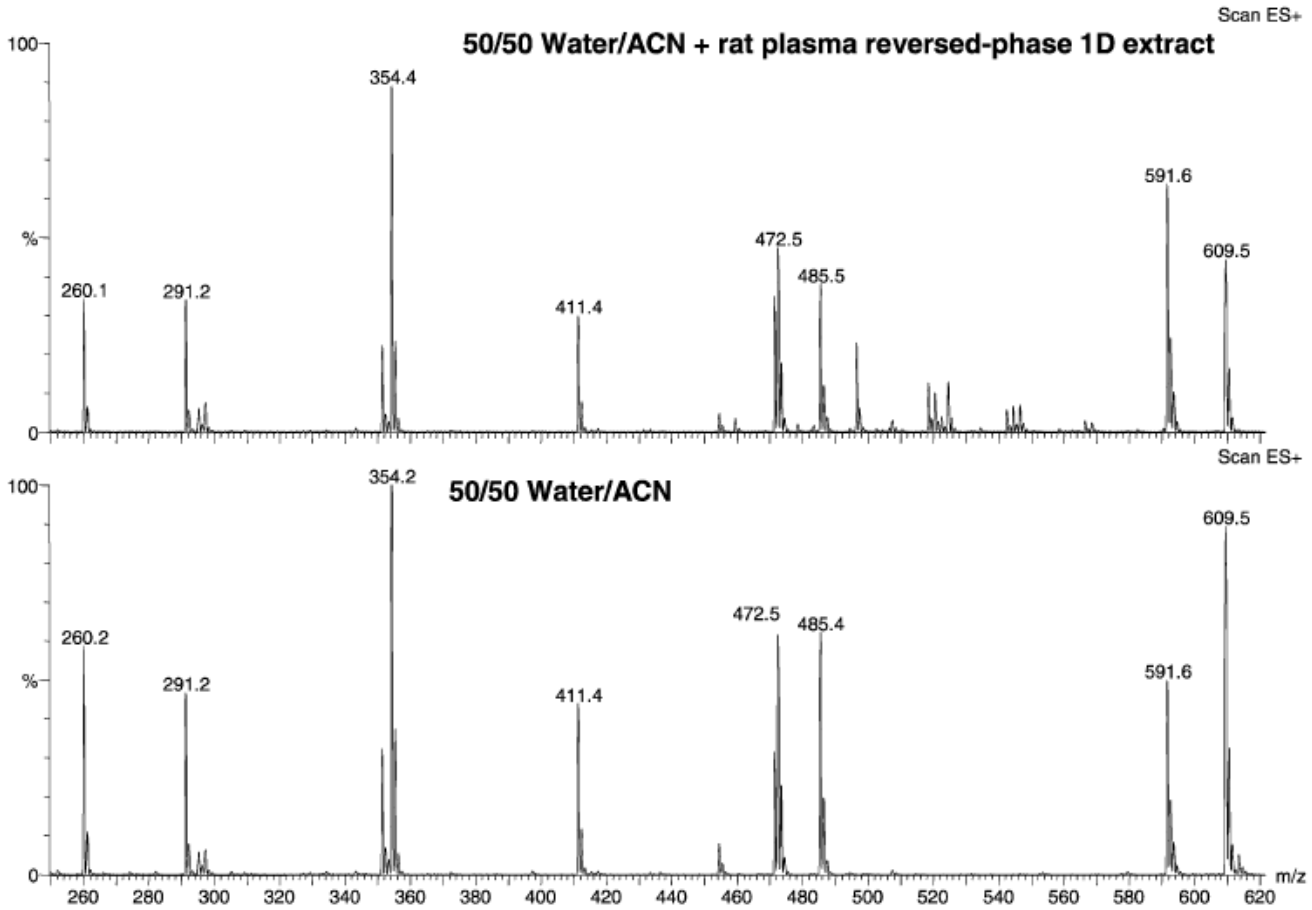


Figure 4. Mass spectra of solution of basic test analytes infused with reversed-phase 1D rat plasma extract compared with result when infused with pure solvent (same scale).

Mallet, C. R.; Lu, Z.; Mazzeo, J. R. *Rapid Communications in Mass Spectrometry* **2004**, *18*, 49–58.

Matrix Effects: Sample Clean-up

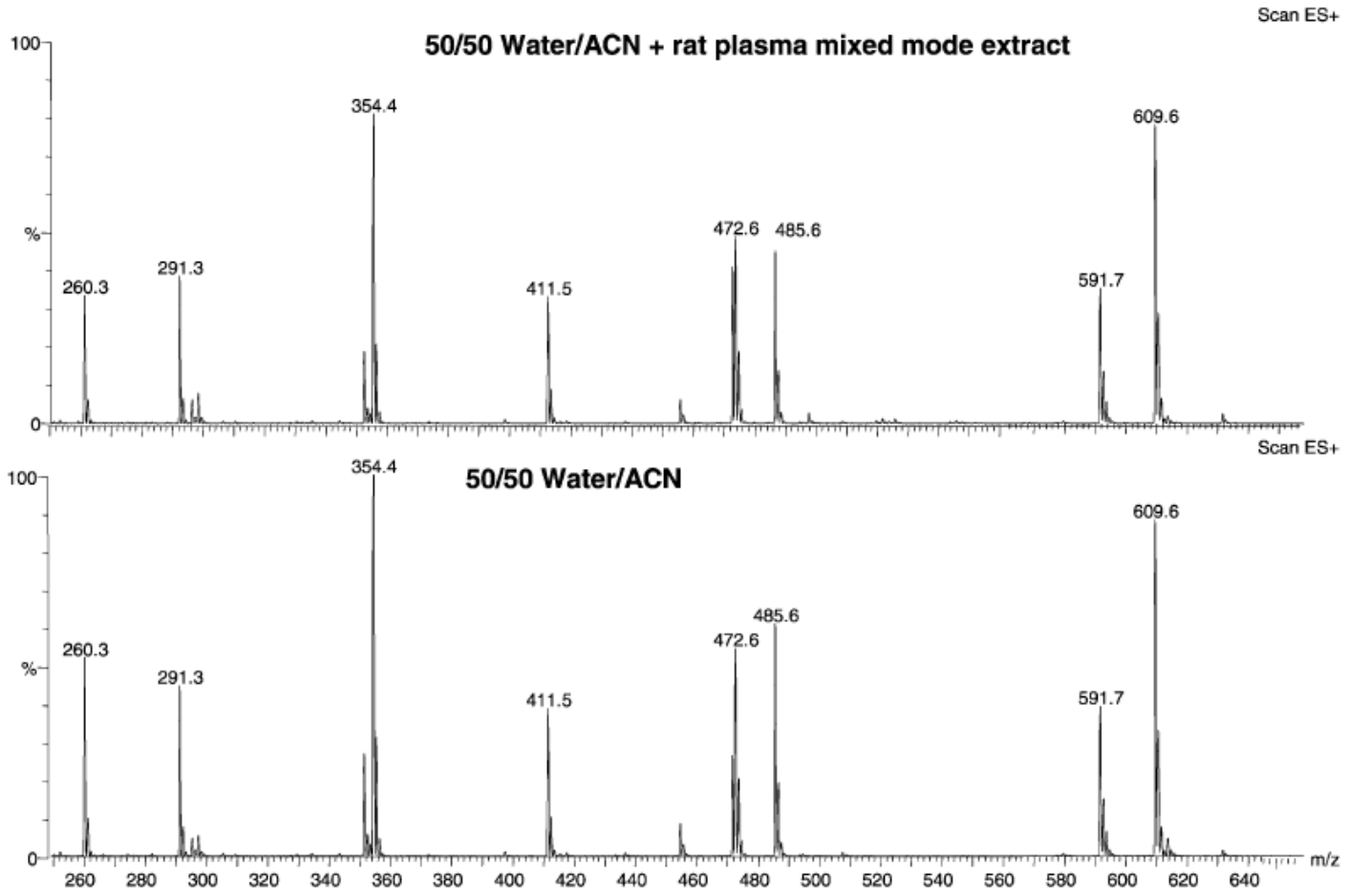
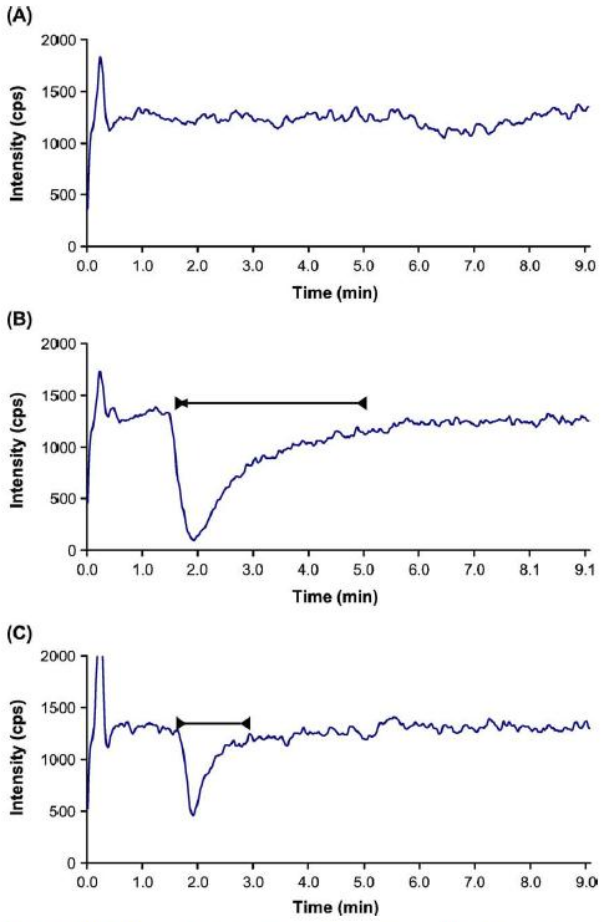


Figure 5. Mass spectra of solution of basic test analytes infused with mixed-mode rat plasma extract compared with result when infused with pure solvent (same scale).

Mallet, C. R.; Lu, Z.; Mazzeo, J. R. *Rapid Communications in Mass Spectrometry* **2004**, *18*, 49–58.

Matrix Effect of Blood and Sample Preparation Method



Baseline

Protein Precipitation

SPE

Fig. 2. Comparison of (A) mobile phase, (B) whole blood sample prepared by protein precipitation, and (C) a whole sample prepared by solid phase extraction [33] by the postcolumn infusion method. The areas influenced by matrix effects are shown in B and C. The solid lines indicate the regions of altered ionization due to matrix effects.

Taylor PJ, Johnson AG. J Chromatogr. B 1998;718:251–7.

Approaches to Reduce Matrix Effects

Table 2
An overview of approaches to reduce matrix effects.

A step of bioanalytical method	ME reduction approach	Examples of realization
Sample preparation	More extensive clean-up	SPE-based approaches with extensive and well optimized washing steps, RAM
		LLE-based approaches – ionized species do not partition into the organic layer
	Higher selectivity	SPE, MIP, immunoaffinity SPE
	Protein precipitation prior to SPE/LLE	
	Dilution of sample	
Chromatography	Higher separation efficiency	Fast/high resolution LC approaches, 2D-LC
	Nano-LC	Nano flow-rates, smaller droplets formed
	Change in selectivity	HILIC or other orthogonal chromatographic mode, change in mobile or stationary phase
	Gradient elution	Change in selectivity, enhancement of efficiency and also elution of highly retained interfering compounds
Mass spectrometry	Higher selectivity	Negative ion mode
	Ionization technique less susceptible to ME	APPI, APCI, EI-MS
Calibration data processing and other strategies	Appropriate calibration approach	Internal standard method, standard addition method, matrix-matched calibration
	Use of SIL-IS	^{13}C SIL-IS should be preferred over deuterium labeled compounds
	Echo peak strategy [122]	Elution very close to t_R of analyzed compounds ~ the same ME

EI-MS (electron ionization mass spectrometry).

Nováková, L. Challenges in the development of bioanalytical liquid chromatography–mass spectrometry method with emphasis on fast analysis. *Journal of Chromatography A* **2012**.

Reduced Matrix Effect by 2D-LC (sLCxLC)

SRM chromatogram from a 1D-LC separation

SRM chromatogram from a sLCxLC separation

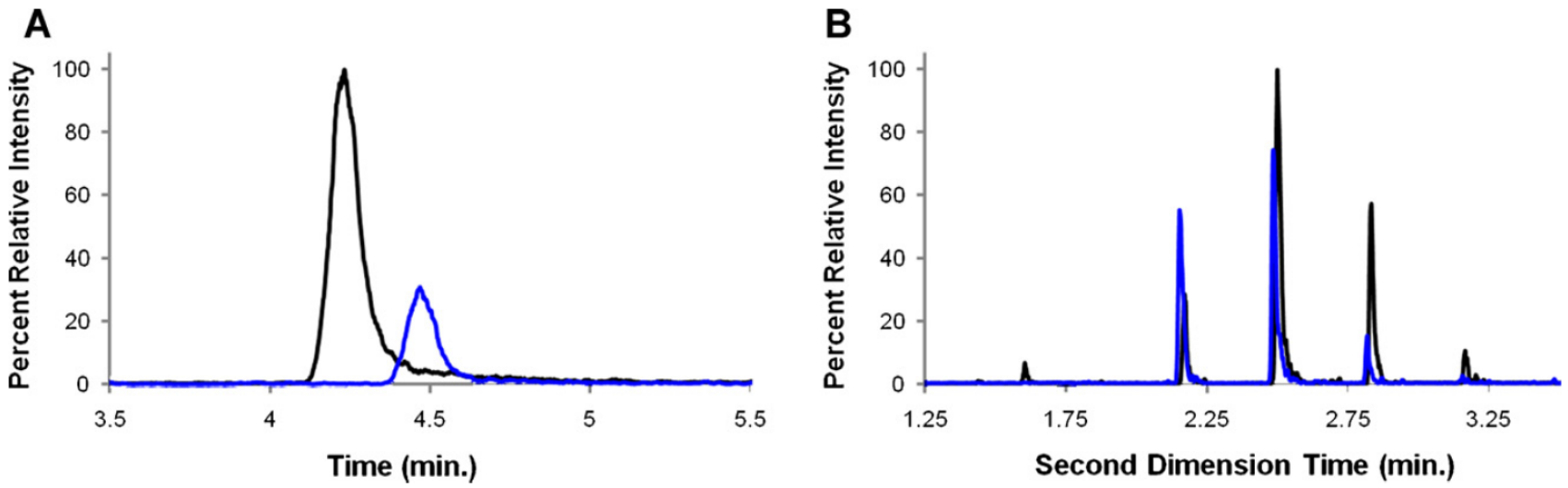


Fig. 7. Comparison of 1D and sLC × LC separations of d_3-cocaine in 1000× concentrated WWTPE using MS/MS detection. Panel (A) shows the SRM chromatogram obtained from a conventional 1D-LC separation, and panel (B) shows the SRM chromatogram obtained at the outlet of the second dimension of the sLC × LC system. The black trace on both panels shows the separation of a 100 ppb spike of d_3-cocaine into DI water and the blue traces are for WWTPE spiked with 100 ppb d_3-cocaine. In the sLC × LC case the sampling window was adjusted to accommodate the ^{1}D retention shift observed in panel (A) such that the two profiles overlap nicely in panel (B) (18 s shift). For detailed chromatographic conditions, see Section 2.3.5.

Groskreutz, S. R.; Swenson, M. M.; Secor, L. B.; Stoll, D. R. *Journal of Chromatography A* **2012**, *1228*, 41–50.

Conclusions

- Acetone might be better than it was shown but definitively a viable alternative
- The choice of the additive is mode (ESI + or -) and sample dependent
- Matrix effects have to be addressed whenever possible
- Biological samples clean-up is highly advisable (almost mandatory…)
- Quality of solvents is very important, especially in Scan mode
- Matrix effects can be reduced with more resolving power

Question:

- How can we improve the baseline in Fast Online 2D-LC?
  - Consistent flow rate
  - Balanced ionization in gradient solvents?
  - Selective background removal method?