

Applicability of High Performance Liquid Chromatography Coupled to Ion Trap Based Mass spectrometry in the Analysis of Endocrine Disrupting Chemicals

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Endocrine disruption:

- Chemicals ? reproduction and development
- Estrogens: PCBs, phthalates, pesticides, contraceptives, ...
- low ppb
- 5 ng/mL estradiol ? sex reversal
- LC/MS analysis



Endocrine disruption: Estradiol: 100
Estrone: 7.3
Estriol: 9.7

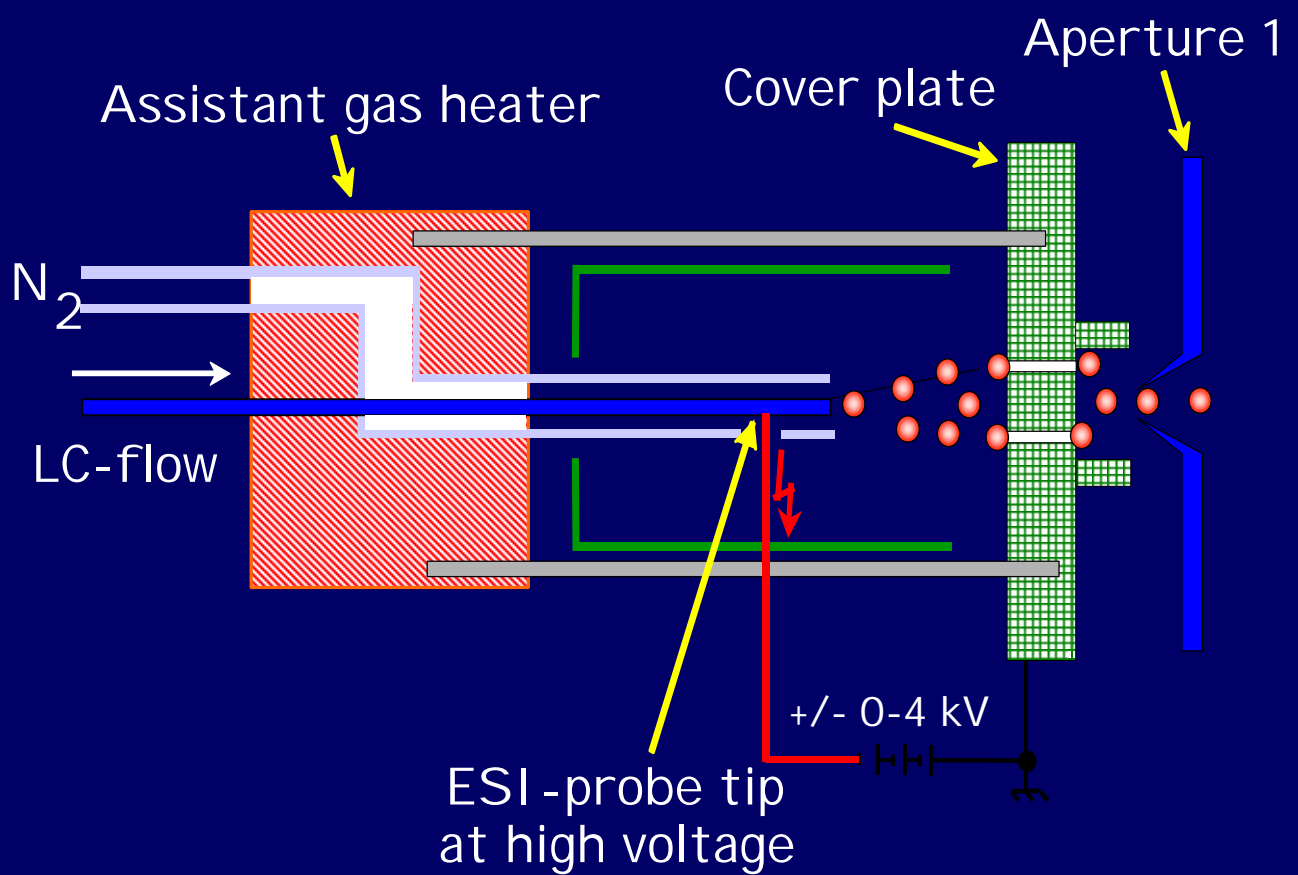
• natural estrogens: Ethynyl estradiol: 140
DES: 400

• pharmaceuticals: 4-octylphenol: 0.005
4-t-octylphenol: 0.015
4-n-nonylphenol: 0.031
• alkylphenols: 4-t-butylphenol: 0.0004
4-t-amylphenol: 0.0005
4-sec-butylphenol: 0.00043

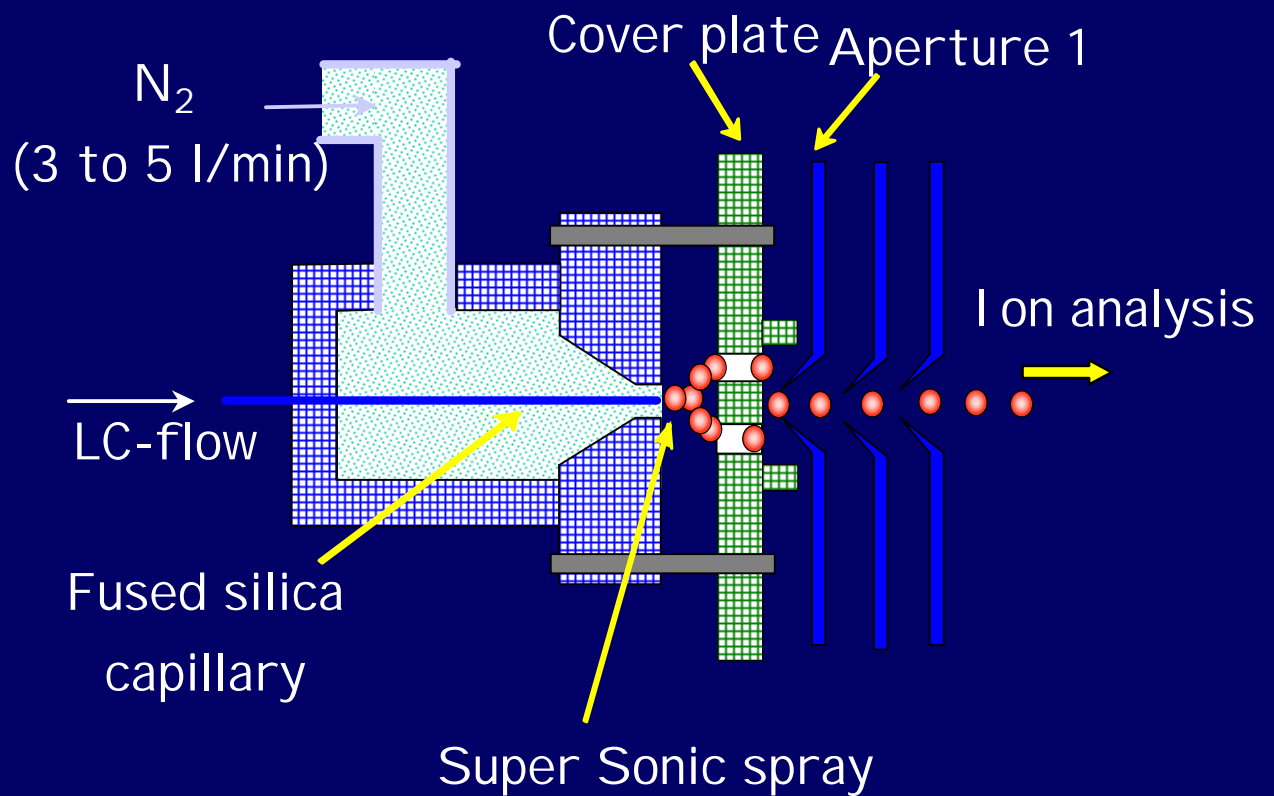
• diphenolalkanes: Cumylphenol: 0.005
Bisphenol A: 0.008
• parabenes: Bisphenol F: 0.0009

• benzophenones: Methylparabene: 0.0004
2,4-dihydroxybenzophenone: 0.002 Ethylparabene: 0.0006
Propylparabene: 0.0006
4,4'-dihydroxybenzophenone: 0.003 Benzylparabene: 0.003

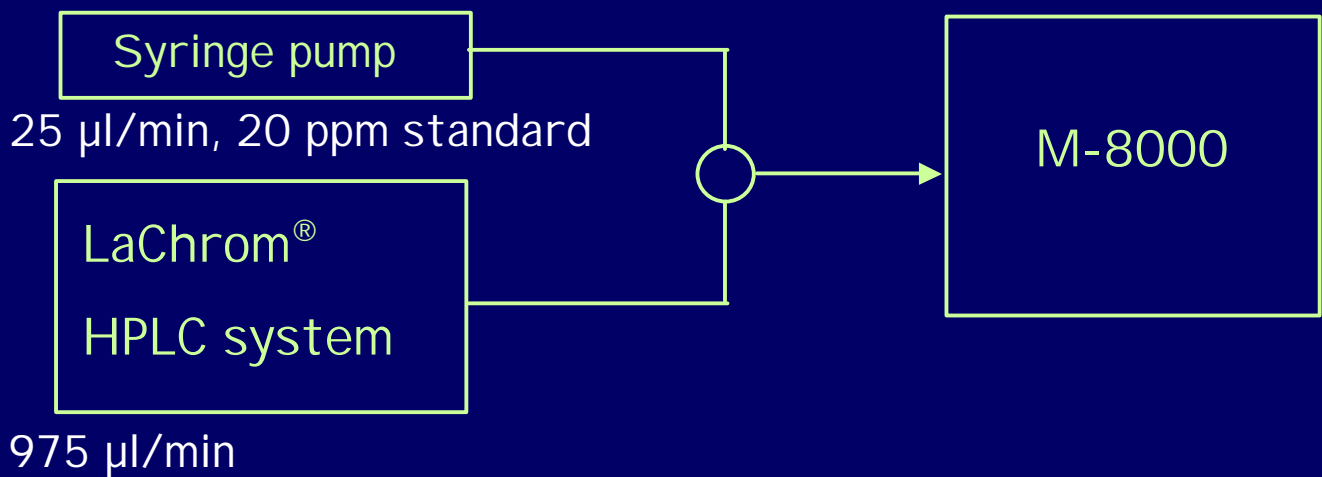
M-8000 ESI ion source



M-8000 SSI ion source



Influence solvent composition

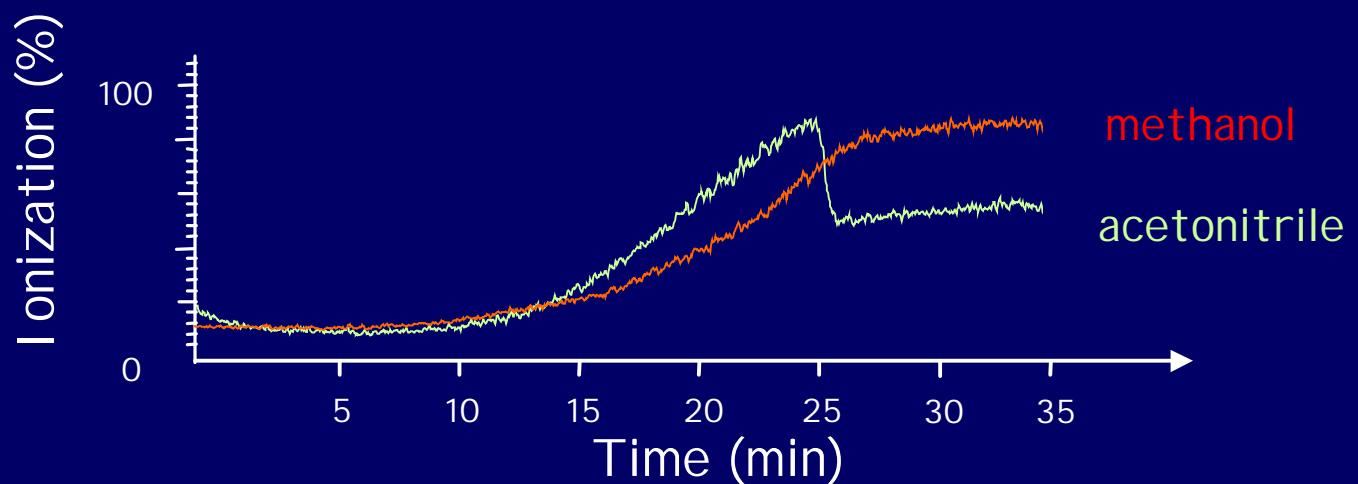


A= starting solvent

B= test solvent

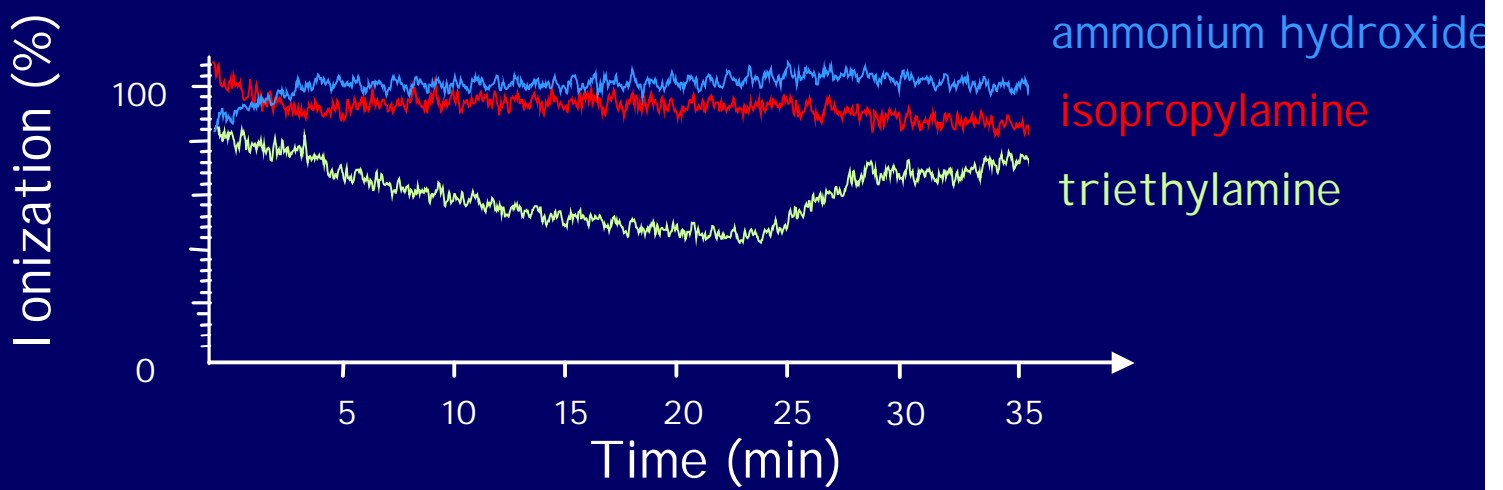
gradient: 0% B ? 100 % B in 20 min.

Influence organic modifier



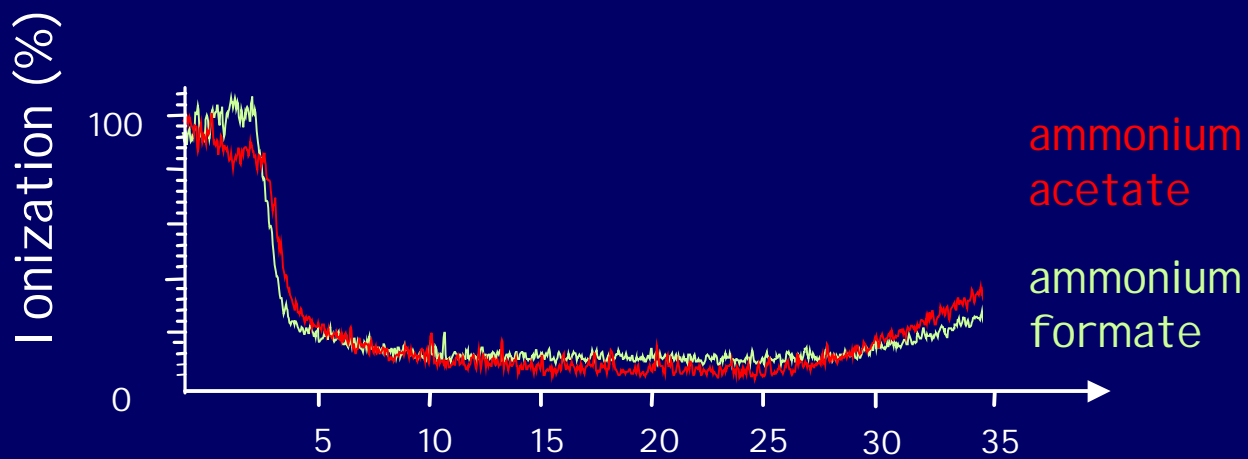
- **Solvent characteristics:** viscosity, conductivity, surface tension
- **Solvating capacities:** MeOH > ACN

Influence volatile bases



- **Ineffectiveness:** analytes deprotonated in solvent
- **Suppression TEA:** ion masking

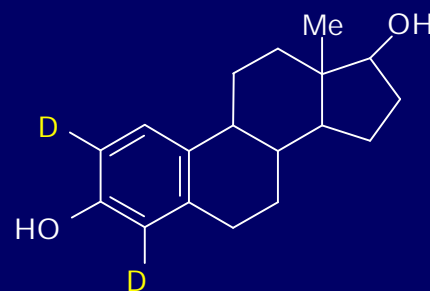
Influence volatile buffers



- Conductivity
- Ion pairing:
positive counter ion? ? ionization suppression

Chromatographic Conditions

- Purospher® Star RP-18 (55 x 4 mm I.D., 3µm)
- Elution profile: gradient
- Flow rate: 1000 µL/min
- Solvents: (A) water
(B) acetonitrile
- Analysis time: 20 minutes
- IS: 1,3,5 (10)-estratriene-2,4-d₂-3,17? -diol



Sample preparation: SPE

- RP-18 (2g, 6 mL)
- 50 mL water (pH 2.5) + I S + 500 μ L MeOH

1. Preconditioning
6 mL MeOH, 10 mL H₂O

2. Sample loading
5 mL/min

3. Elution
dichloromethane/i-propanol (80:20, v/v)

4. Drying under N₂ and redissolving
50 μ L acetonitrile/water (80:20, v/v)



Recoveries: Tap water spiked at 10 ng o.c. (n = 5)

analyte	m/z	Recovery	CV%
estradiol	271	101.9	11.3
estrone	269	106.5	7.4
estriol	287	112.6	13.5
ethynyl estradiol	295	88.6	9.5
DES	267	110.5	6.5
IS	273	103.4	11.1
4-t-octylphenol	205	81.5	14.3
4-octylphenol	205	76.3	13.2
4-t-butylphenol	149	101.4	10.5
4-sec-butylphenol	149	113.4	9.3
4-t-amylphenol	163	84.8	12.8
4-n-nonylphenol	219	86.1	9.8
4-cumylphenol	211	83.7	8.1
bisphenol A	227	85.7	10.6
bisphenol F	199	103.7	8.6
methylparabene	151	101.6	5.2
ethylparabene	165	90.0	6.1
propylparabene	179	96.1	5.6
benzylparabene	227	104.4	7.0
2,4-dihydroxybenzophenone	213	86.1	11.6
4,4'-dihydroxybenzophenone	213	97.2	9.1

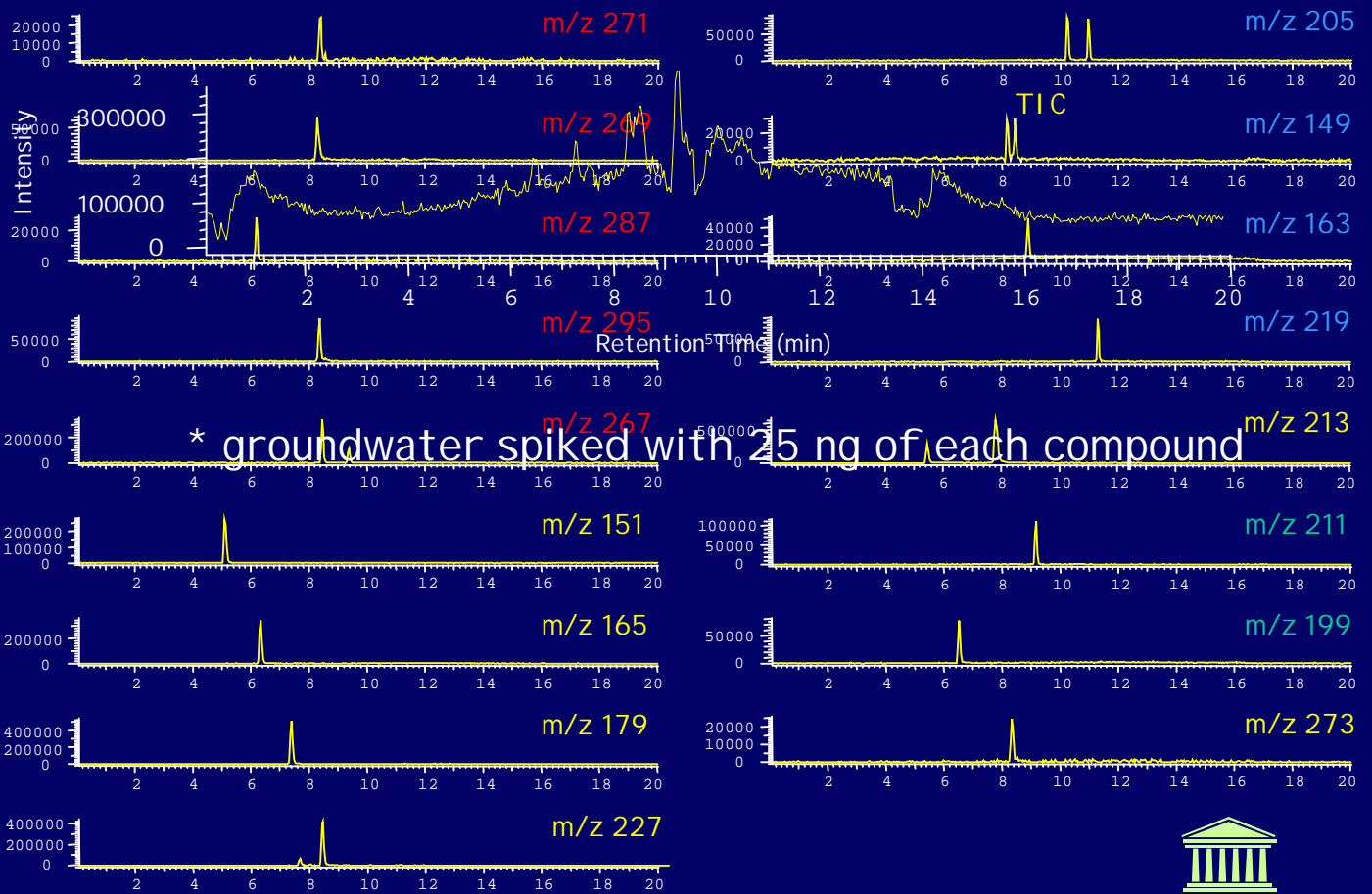


Analytical performance:

- LOD: 3.2 ppt – 10.6 ppt
- LOQ: 9.6 ppt – 31.8 ppt
- Linearity: $0.9870 < R^2 < 0.9997$
- Within-day reproducibility (n=5): 5.2% - 14.3%
- Between-day reproducibility (n=5): 9.4% - 18.1%



Real samples:



Conclusion

- novel API : SSI
- 21 estrogenic compounds
- SPE
- Environmental water samples
- Linear
- Sensitive
- Robust



Acknowledgements

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