



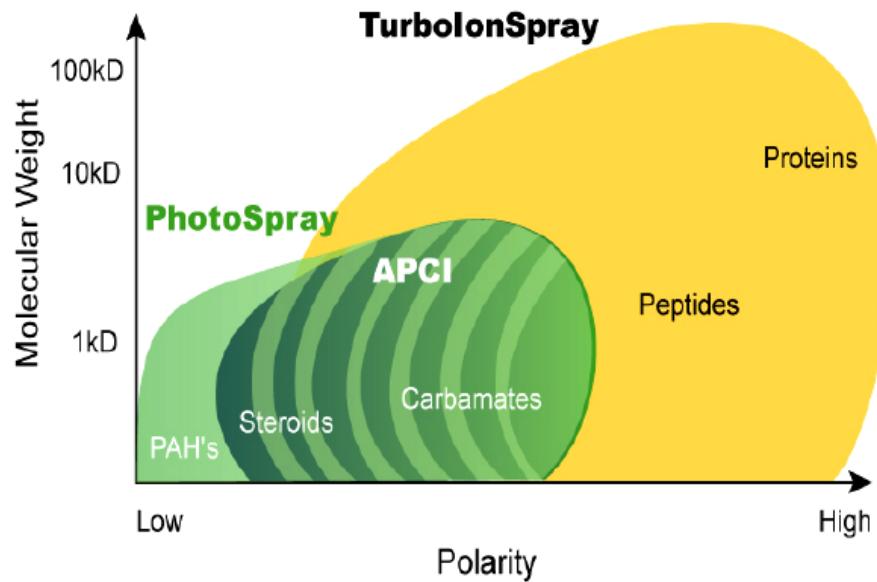
Advantages and practical use of photo- spray ionization for quantitative bioanalysis

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PRA International
12 June 2012

What is photo ionization?

Atmospheric pressure photoionization (APPI):

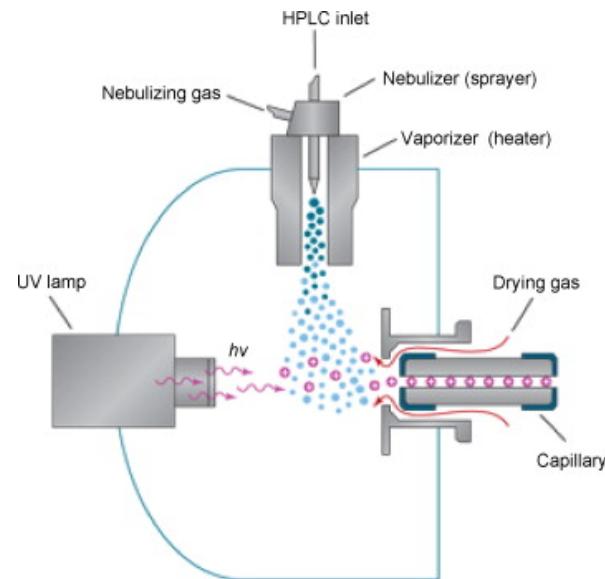
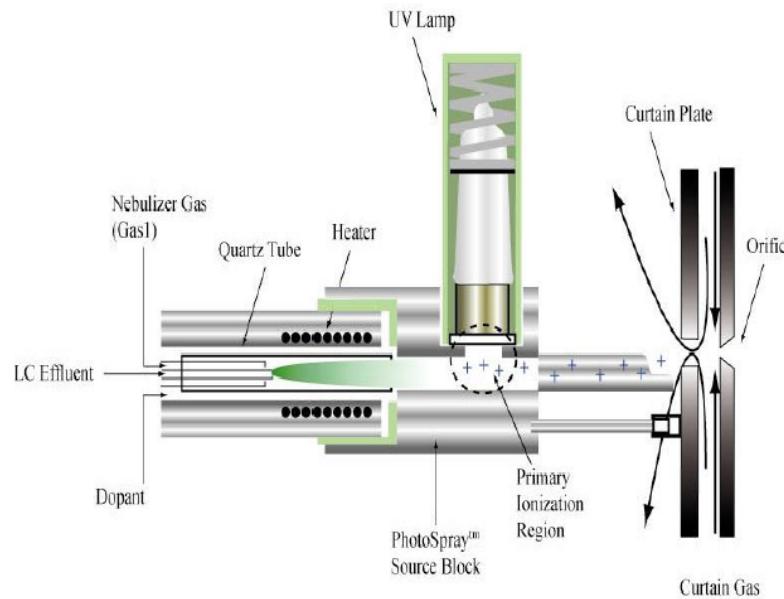
Complementary ionization technique, introduced in the 1990s.
Commercial interfaces available since 2001



> 50% of bioanalytical applications of APPI are with steroids

Review paper: Bos et al., Anal. Bioanal. Chem., 384 (2006) 85.

What is photo ionization?





What is photo ionization?

Principles of APPI



if photon energy > ionization energy

Most analyte molecules have ionization potential (IP)

< 10 eV

Most LC solvents have IP > 10 eV

Selective ionization of analyte in eluent with 10 eV lamp



What is photo ionization?

Photoionization via dopant (D):



if electron affinity D > electron affinity M

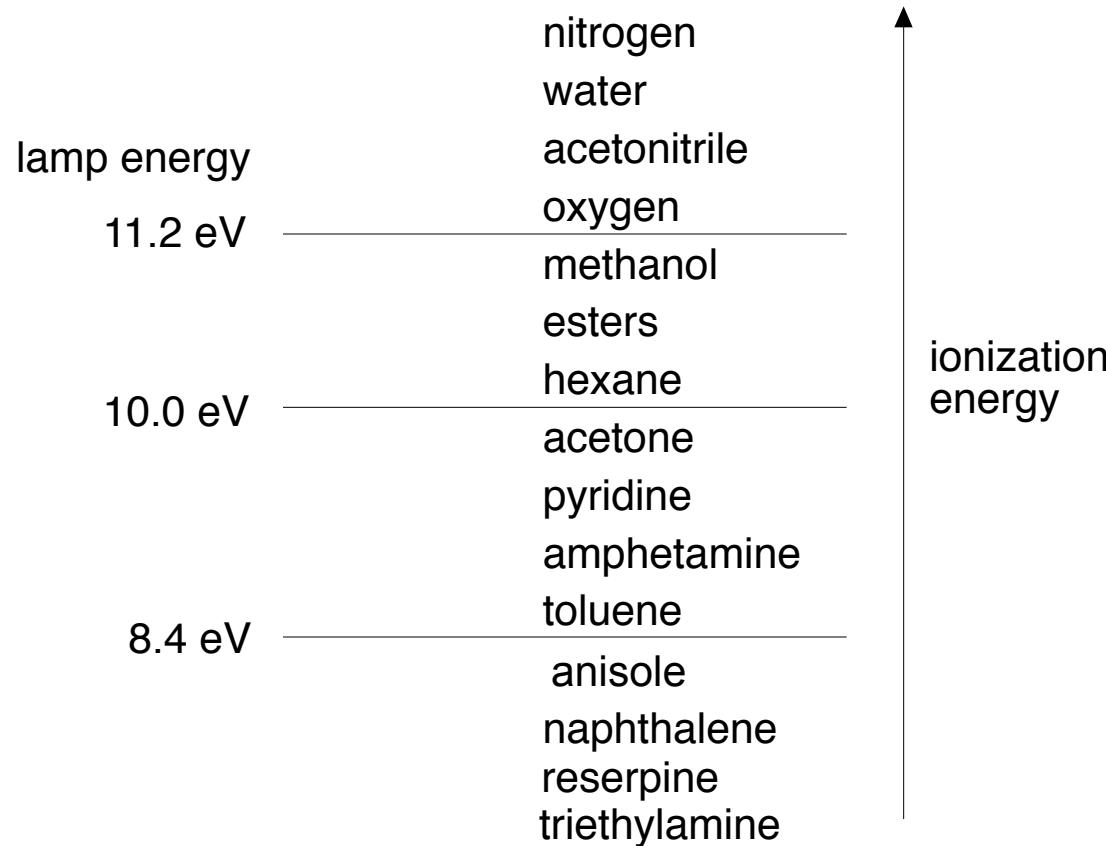
Common dopants: acetone IP 9.7 eV

toluene IP 8.8 eV

anisole IP 8.2 eV

What is photo ionization?

Photoionization Lamps and Ionization Energies



What is photo ionization?

Photoionization via dopant (D) and solvent (S):



if proton affinity M > proton affinity S > proton affinity D

Most common mechanism in LC-APPI-MS

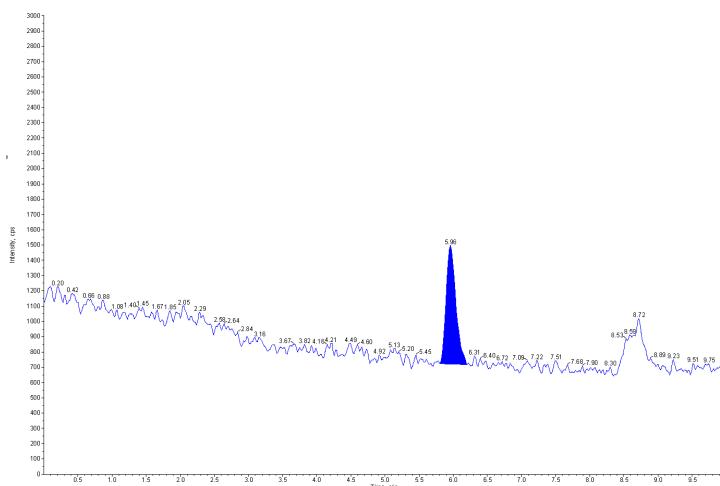
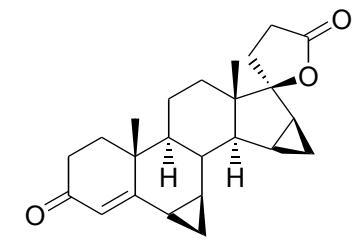
ca 80% of bioanalytical applications of APPI use toluene and
[M+H]⁺

APPI is sensitive to mobile phase composition !

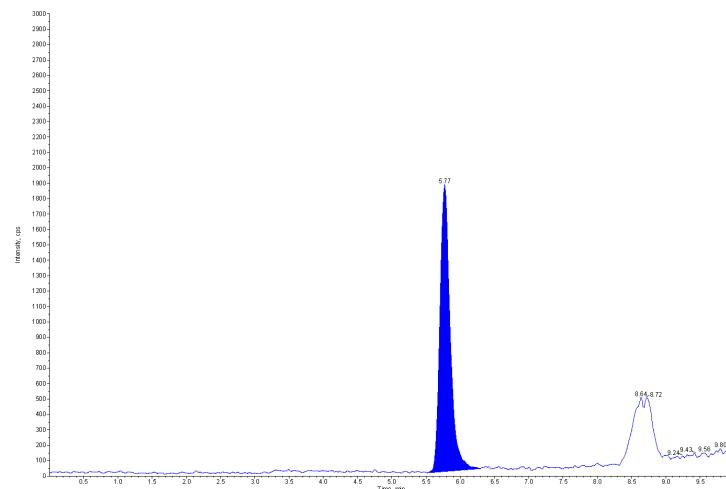
Example 1: APCI vs APPI

Sensitivity gain

test solution drospirenone (1 ng/ml, API 3000)



APCI

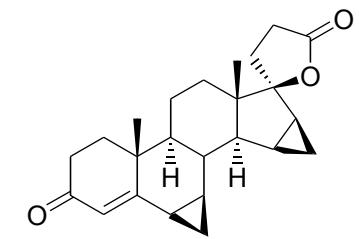


APPI / toluene

Example 2: Effect of dopant

Use of dopant

test solution drospirenone (1 ng/ml, API 3000)



APPI / no dopant

APPI / toluene

Example 3: application

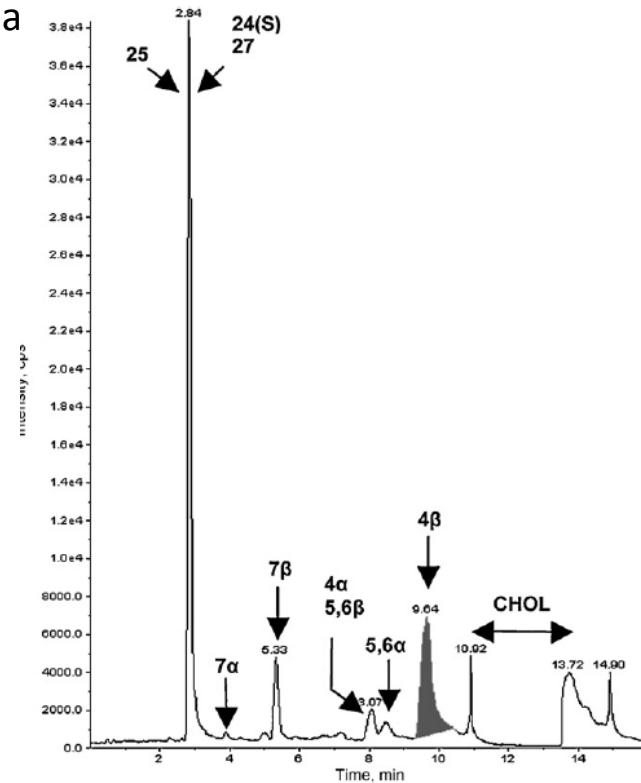
Determination of 4β -hydroxycholesterol in human plasma

Separation needed from other isobaric oxysterols

Normally performed with GC-MS after overnight derivatisation.

10 cm C18 column with 2.2 μ m particles
API 3000 with APPI and toluene as dopant

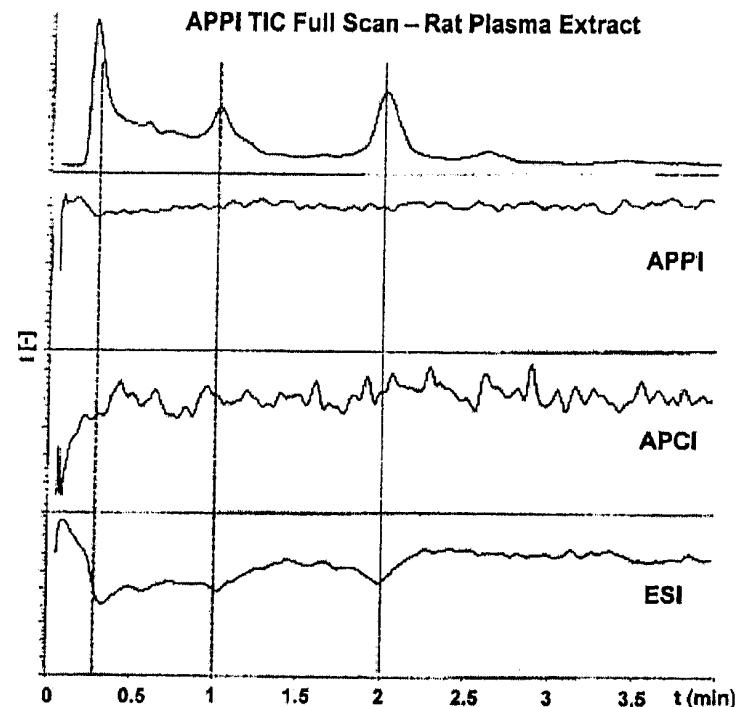
Limit of quantitation = 10 nM



N.C. van de Merbel et al. / J. of Pharm. and Biomed. Anal. 55 (2011) 1089–1095

Example 4: Ion suppression

Ion suppression susceptibility:
post-column infusion of
fluphenazine



Hanold et al., Anal. Chem., 76 (2004) 2842.

Negative mode APPI

Other mechanisms:

Negative mode APPI / dopant

Photoionization via dopant (D) and solvent (S):



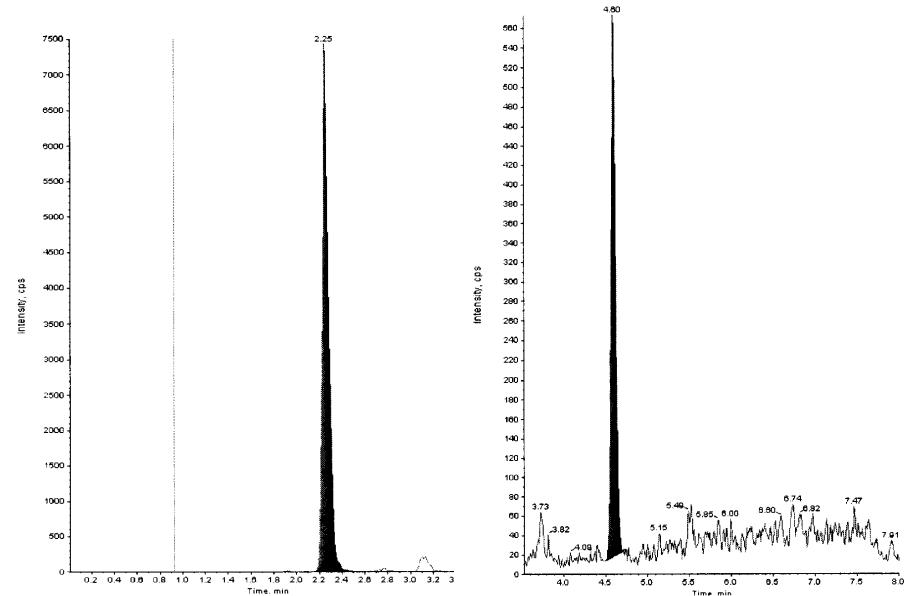
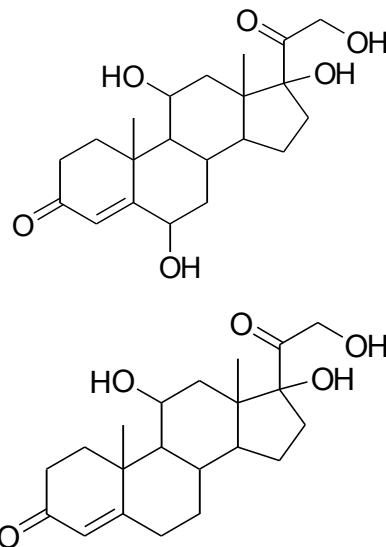
for analytes with gas-phase acidity

Example 5: negative mode

Negative mode APPI / toluene

6 β -hydroxycortisol: [M+formiate] $^-$

cortisol: [M+H] $^+$

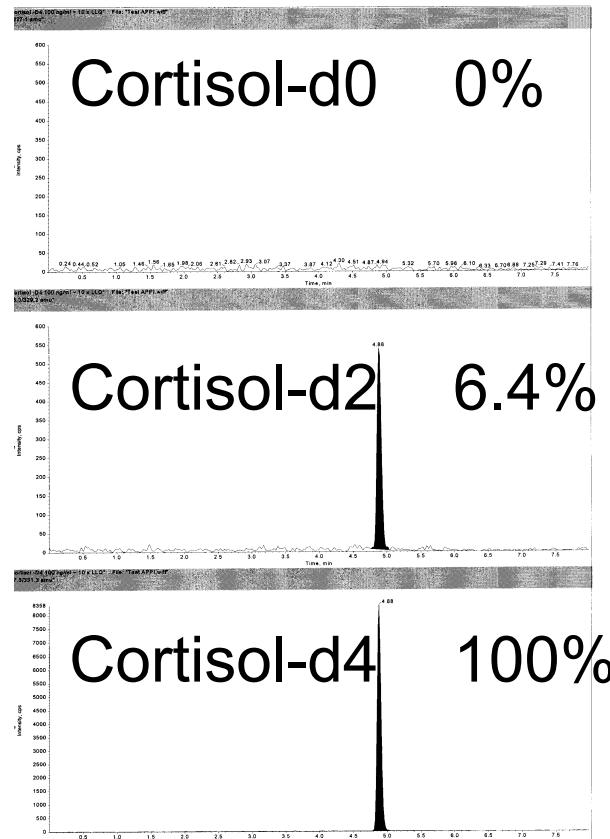


6 β -OH: 5 ng/ml

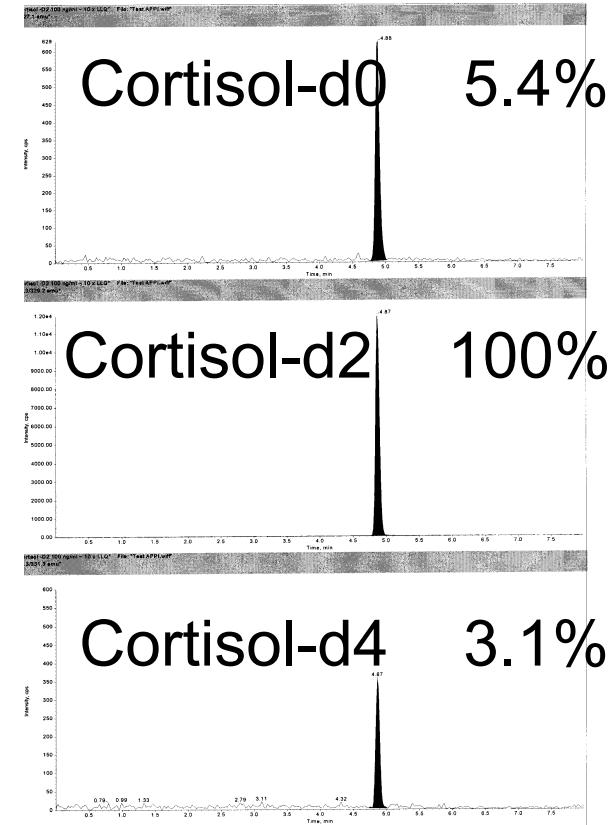
cortisol: 1 ng/ml

IS consideration

Observation: loss of 2 amu during APPI



Test solution cortisol-d4



Test solution cortisol-d2

Dopant free APPI

Other mechanisms:

Dopant-free APPI

Photoionization via solvent (S) only:



Dopant free APPI: Example 6

Simultaneous determination of biomarkers for cholesterol homeostasis in rat and human:

Synthesis:

Lanosterol

Intestinal absorption:

β -sitosterol:

Elimination:

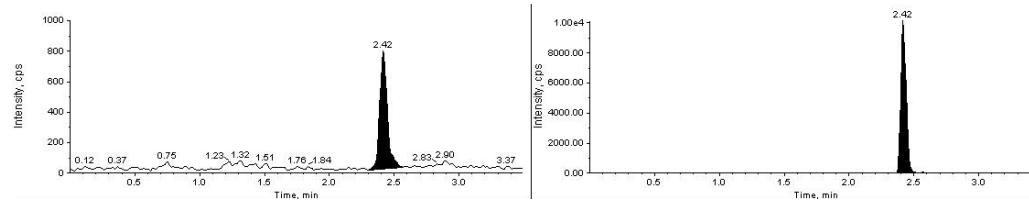
4-cholestene-7 α -ol-3-one

Challenges: single method, low sample volume, nM LLOQ needed, sterols have relative low proton Affinity (limits mobile phase choice).

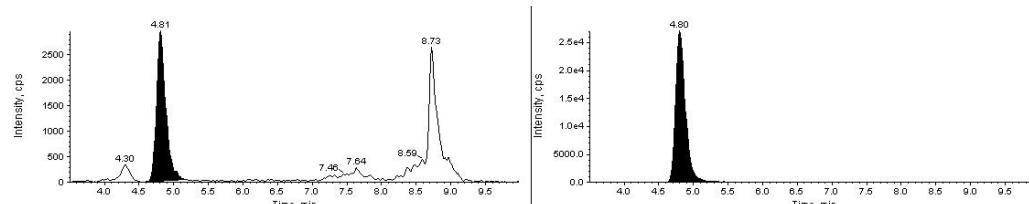
Dopant free APPI

Ultracarb ODS 30 Column using mainly butanol and hexane

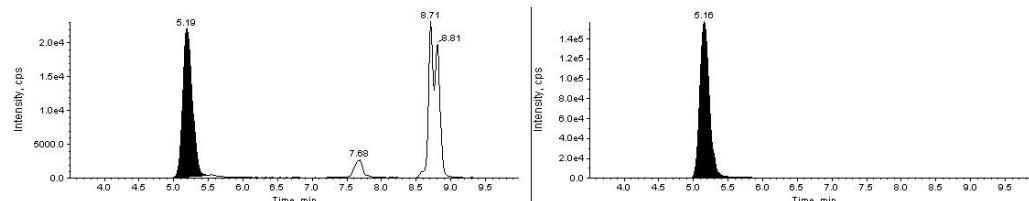
4-cholestene-7 α -ol-3-one
(12.2 nM)



Lanosterol
(170 nM)



β -sitosterol
(1713 nM)



Submitted for publication



Conclusions

APPI has improved the possibilities to measure low levels of non-polar compounds replacing older techniques

APPI can prevent the need for labor intensive derivatisation

Sensitivity largely dependent on right dopant, flow rate, eluens and additives

Do not use internal standards with a mass difference of 2 amu

Important consideration: How does the sensitivity relate to the latest ESI-MS instrumentation???