Pitfalls associated with the use of a simple sample preparation in the analysis of saliva with LC-ESI-MS/MS

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Introduction: LC-MS in STA

Routine analysis of toxicological samples:

- Screening:
  - immunological techniques (e.g. EMIT)
- Confirmation:
  - chromatographic techniques (e.g. HPLC-DAD or Fl; GC-MS)
  - ⇨ LC-MS as valuable alternative

Objectives: fast and easy analysis

Simple sample preparation:

- Enrichment:
  - Not strictly necessary
    LC-MS is very sensitive
- Purification:
  - Saliva is relatively clean

⇨ LC-ESI-MS with protein precipitation

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- Amphetamines
  - Amphetamine, Methamphetamine,
    MDA (3,4-Methylenedioxyamphetamine)
    MDMA (3,4-Methylenedioxymethylamphetamine)
    MDEA (3,4-Methylenedioxyethylamphetamine)
- Opiates
  - Morphine
    Codeine
    6-MAM (6-monoacetylmorphine)
- Cocaine and Benzoylecgonine

Internal standards:

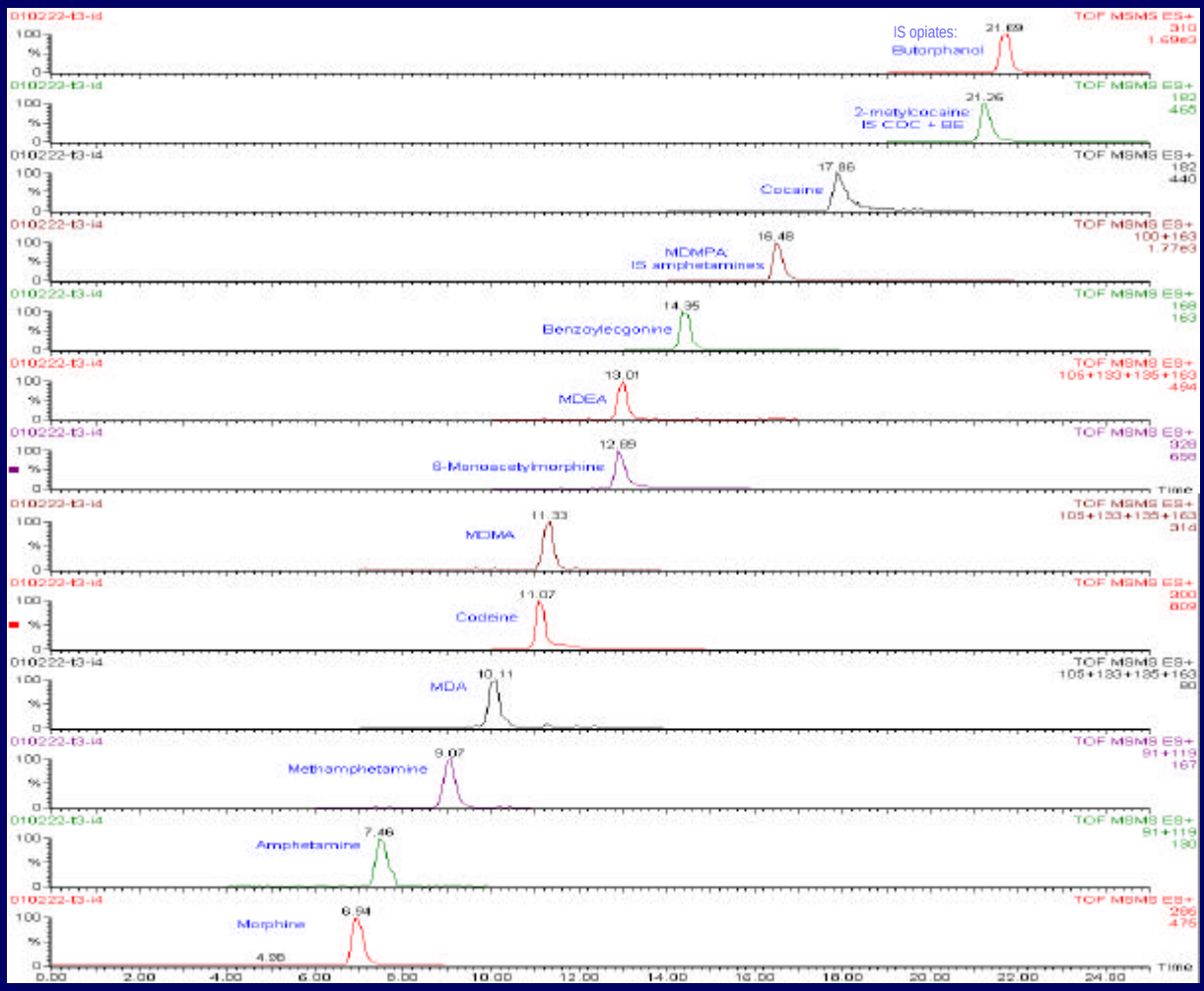
- Amphetamines
  - MDMPA (Methylendioxymethylpropylamphetamine)
- Opiates
  - Butorphanol
- Cocaine and Benzoylecgonine
  - 2’-methylcocaine

Overview of the experiments:

- Sample preparation:

  200 µL saliva (frozen/thawed)
  100 µL of 55% MeOH in water
    - containing IS and standard
  50 µL formic acid
  vortexing - centrifugation
  supernatant pH adjusted with NH_4OH
  50 µL injected

- Chromatographic parameters:
  - Phenyl type column
    Narrow bore, Hypersil BDS
  - Gradient elution
    50 mM NH_4Formate pH 4
    Gradient from 6 to 50% MeOH
    Flow: 200 µL/min Run time: 24’
- Mass spectrometric analysis: MS/MS
  Quantification on fragment ions
  Except opiates: extensive fragmentation



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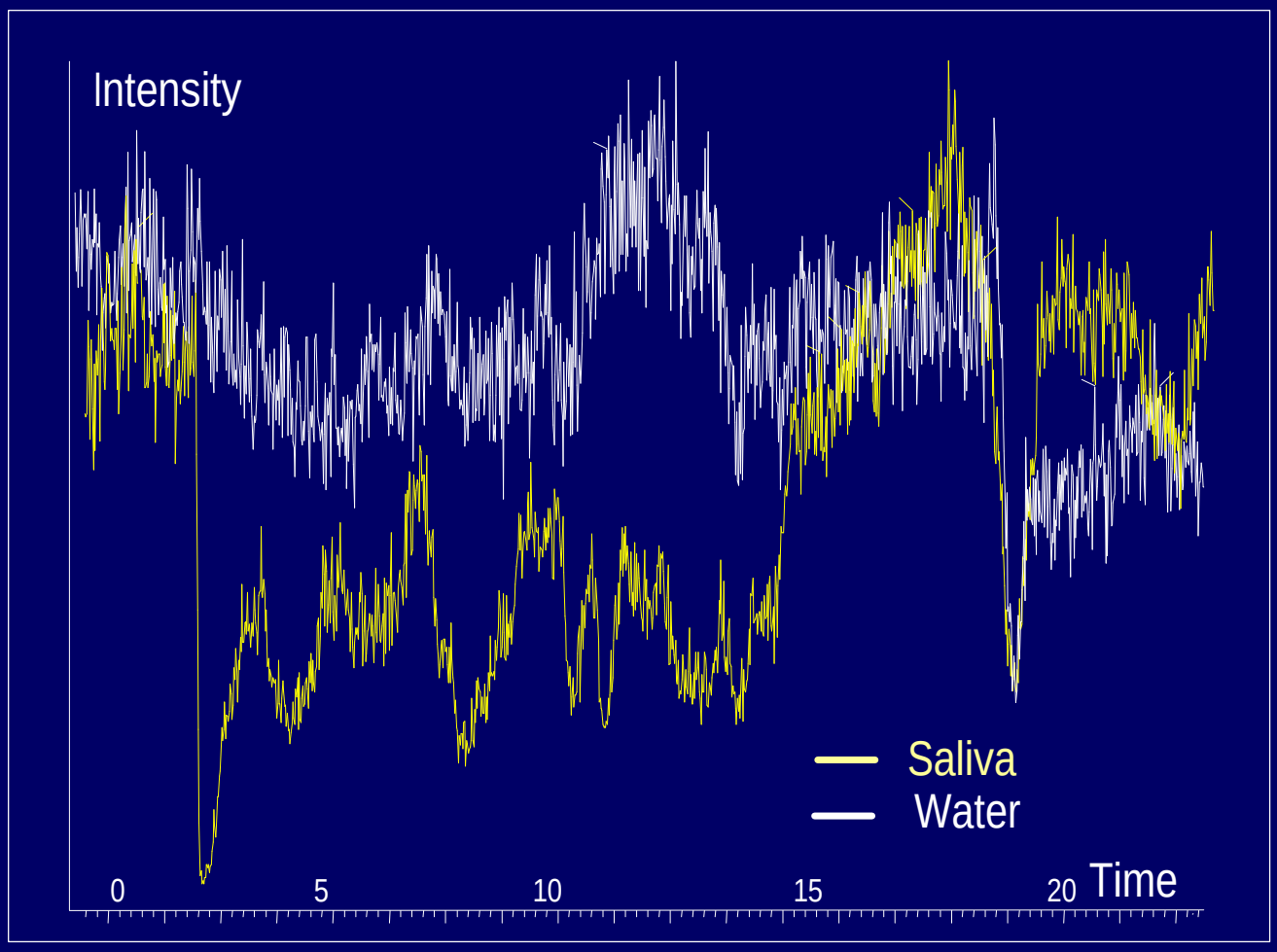
- Validation parameters:
  - Linearity (n=4): 5 - 500 ng/mL
    7 points - $R^2 > 0.995$
  - Within Day Precision (n = 7):
    at 25 ng/mL
    CV% < 10 except MDA = 12.5
  - Total Precision (n = 4):
    For all concentrations
    CV% < 15 except for lowest point
  - Stability: all components stable
  - Accuracy: not acceptable
    sometimes > 20% deviation

Problems and explanation:

- Validation parameters:
  - Matrix suppression: decreased ionization efficiency in ESI
    - saliva of different individuals spiked after sample preparation
    - variable suppression
  - Confirmation of matrix effect
    - post-column introduction of MDMA



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More aggressive protein precipitation

1. 100 µL saliva (frozen/thawed)
   100 µL of pure MeOH
   - containing IS and standard

   500 µL Acetonitrile
   20 µL Methanolic hydrochloric acid
   vortexing - centrifugation - evaporation
   residue redissolved in eluent
   50 µL injected
2. Extra Ultrafiltration step (3000 Da filter)

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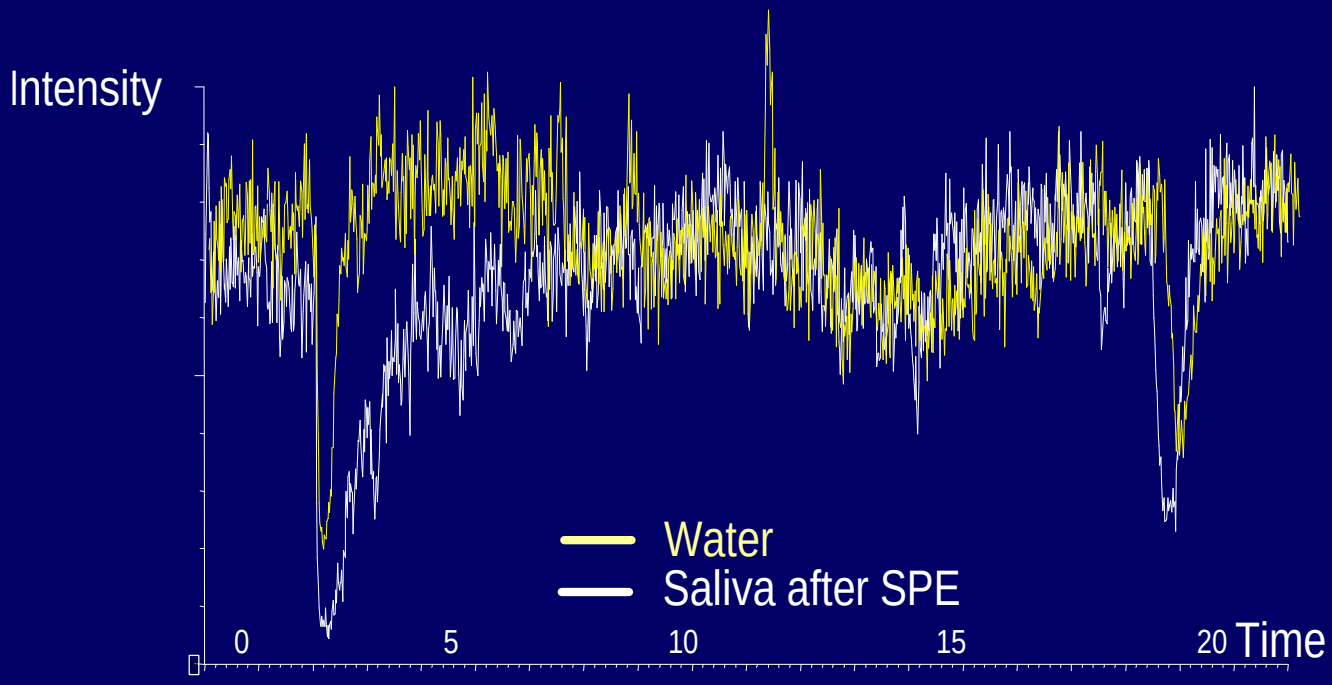
Explanations and tips

- Same problems with SPE?



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- More thorough sample clean-up (e.g. SPE)
- Use of APCI ?
- Use of labelled internal standards?
  - Same retention time
  - Same matrix suppression

Conclusion:

- Pitfall associated with ESI combined with MS/MS

- You only see what you are looking for!