The Role of Liquid Chromatography-Mass Spectrometry in Forensic Toxicology

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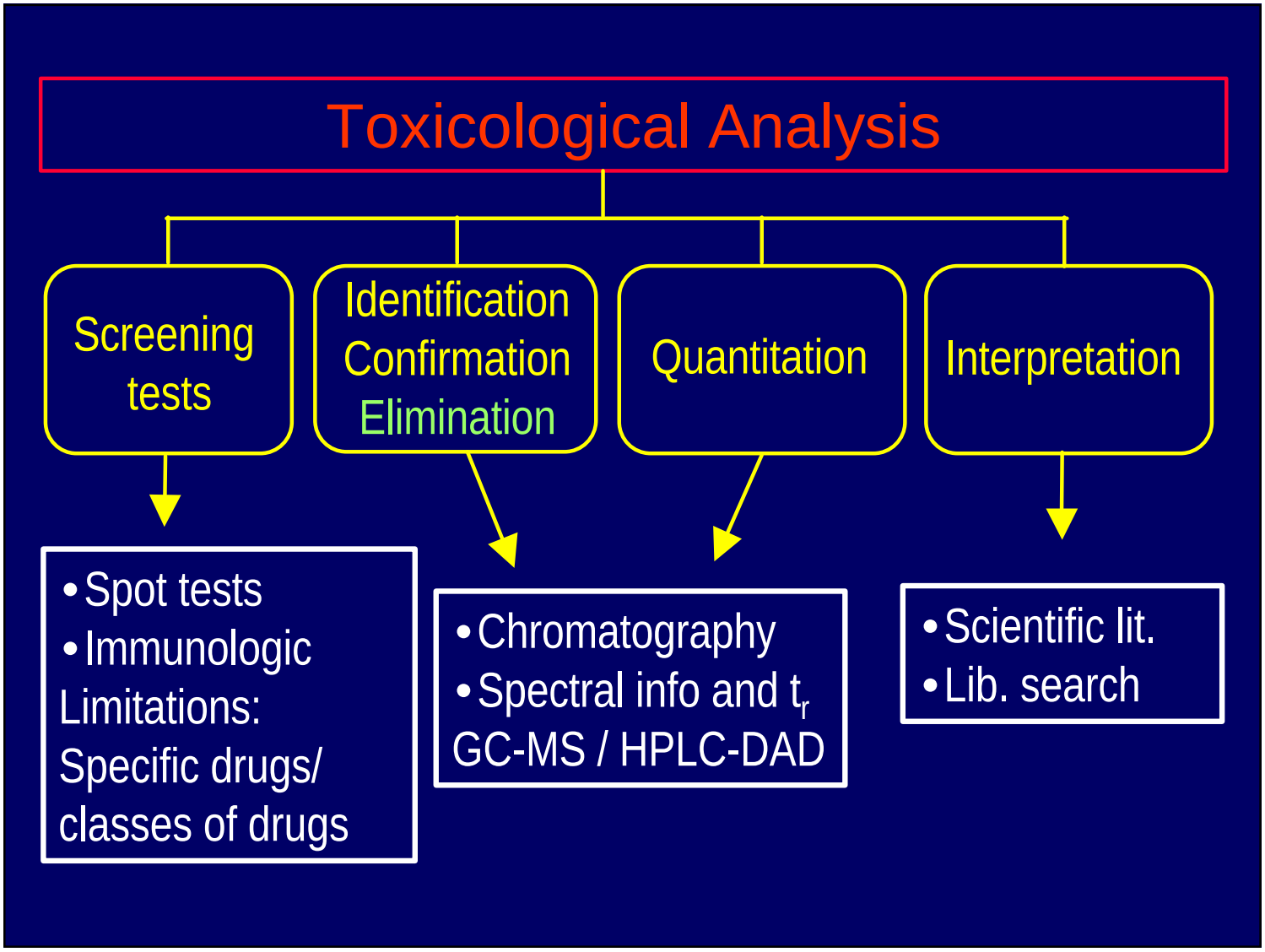
Overview

- Introduction
  - GC-MS LC-DAD
- Interface characteristics
- Applications
  - Opiates / non-classical opiates
  - Cocaine
  - LSD
  - Amphetamines / designer drugs
  - Cannabinoids
  - Multicomponent analysis
- General conclusions


Toxicological Analysis
Screening tests
Identification
Confirmation
Elimination
Quantitation
Interpretation
• Spot tests
• Immunologic
Limitations:
Specific drugs/
classes of drugs
• Chromatography
• Spectral info and t_r
GC-MS / HPLC-DAD
• Scientific lit.
• Lib. search

GC-MS and HPLC-DAD

	Advantage	Disadvantage
GC-MS:	Specific Sensitive Large libraries	Polar Thermolabile High MW compounds
HPLC-DAD:	Polar Thermolabile	Less specific Less sensitive

HPLC-MS: IDEAL COMBINATION?

Interfaces

- Direct Liquid Introduction
- Moving Belt
- Particle Beam
- Thermospray
  - → Limited use

Atmospheric Pressure Ionization (API)

- Electrospray (or Ionspray)
- APCI
- Sonic Spray Ionization
  - → Number of applications increasing

Electrospray

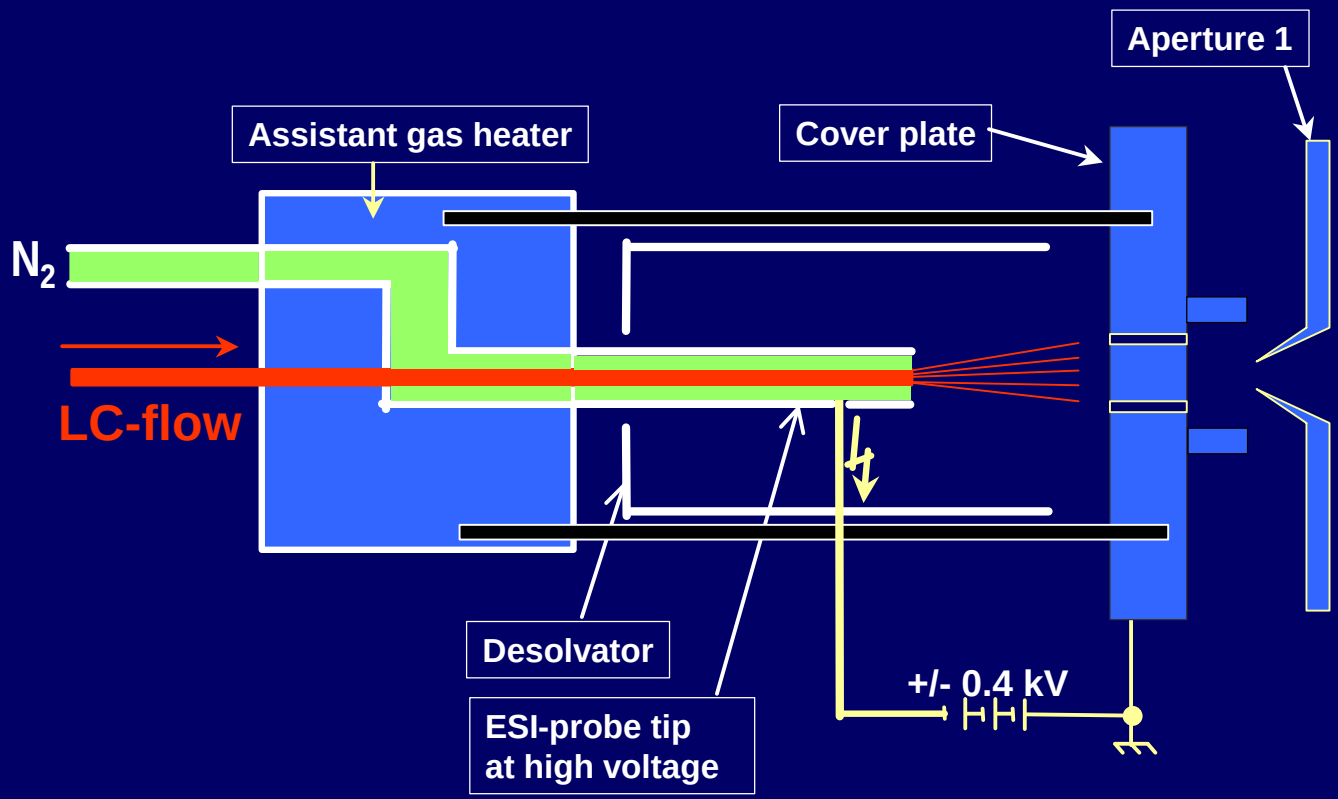
Or with coaxial N_2 flow : Ionspray

- Electric field on capillary sprayer tip
- Ionization in liquid phase
- Concentration sensitive

  → miniaturization

  - Nano-ES : protein, peptide analysis
    Multiple charged ions
  - Capillary electrophoresis
- Soft ionization technique : little fragmentation
- High mol. mass range by multiple charging
- Thermolabile / highly polar compounds

Electrospray mechanism (M-8000, Merck-Hitachi)



Atmospheric Pressure Chemical Ionization

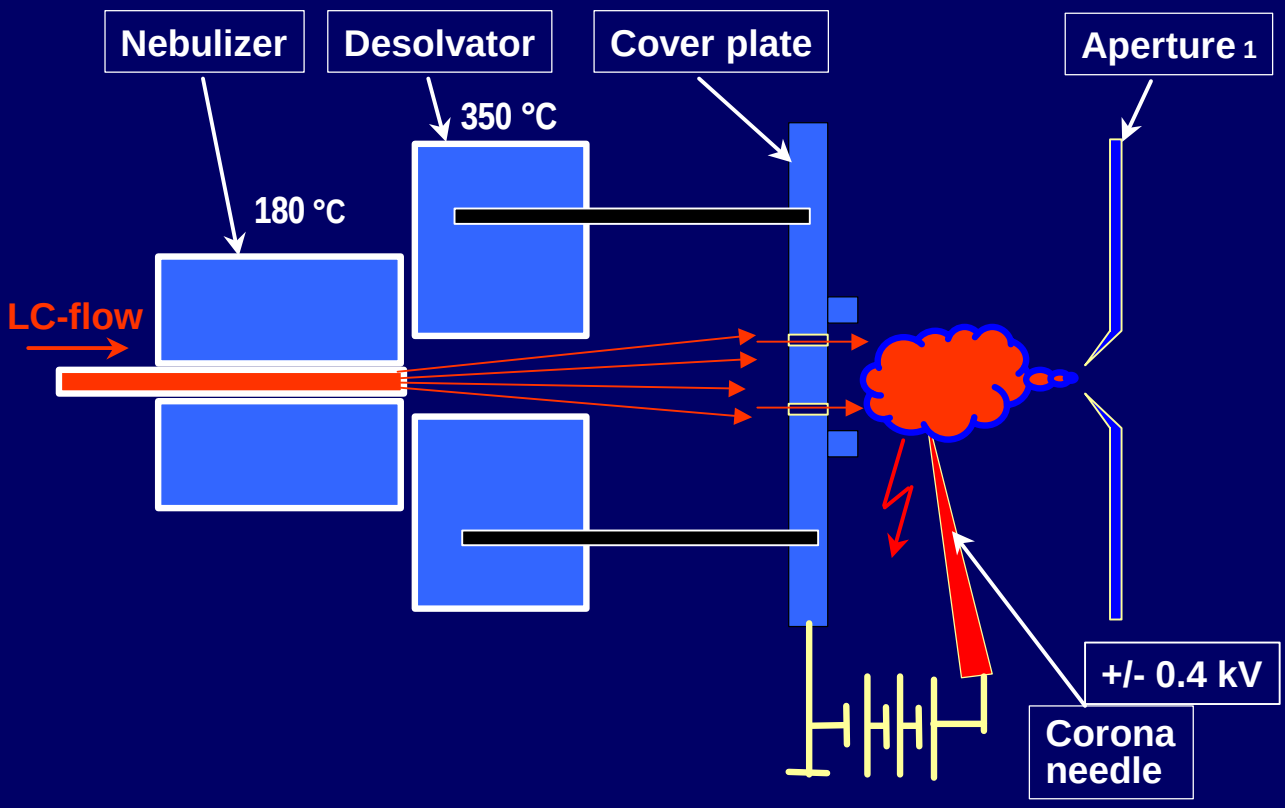
- Higher flows compatible
  Stability of corona discharge
- Ionization in gas phase (analyte / solvent)
- Heated nebulizer (350 – 500°C)
- High ionization efficiency

Most applications:

- Mw < 1000 Da
- Medium to low polarity molec. (not thermolabile)

- Pesticides, drugs, steroids
- High flow rates - standard columns
- Less susceptible to matrix suppression

APCI mechanism (M-8000, Merck-Hitachi)



Sonic Spray Ionization

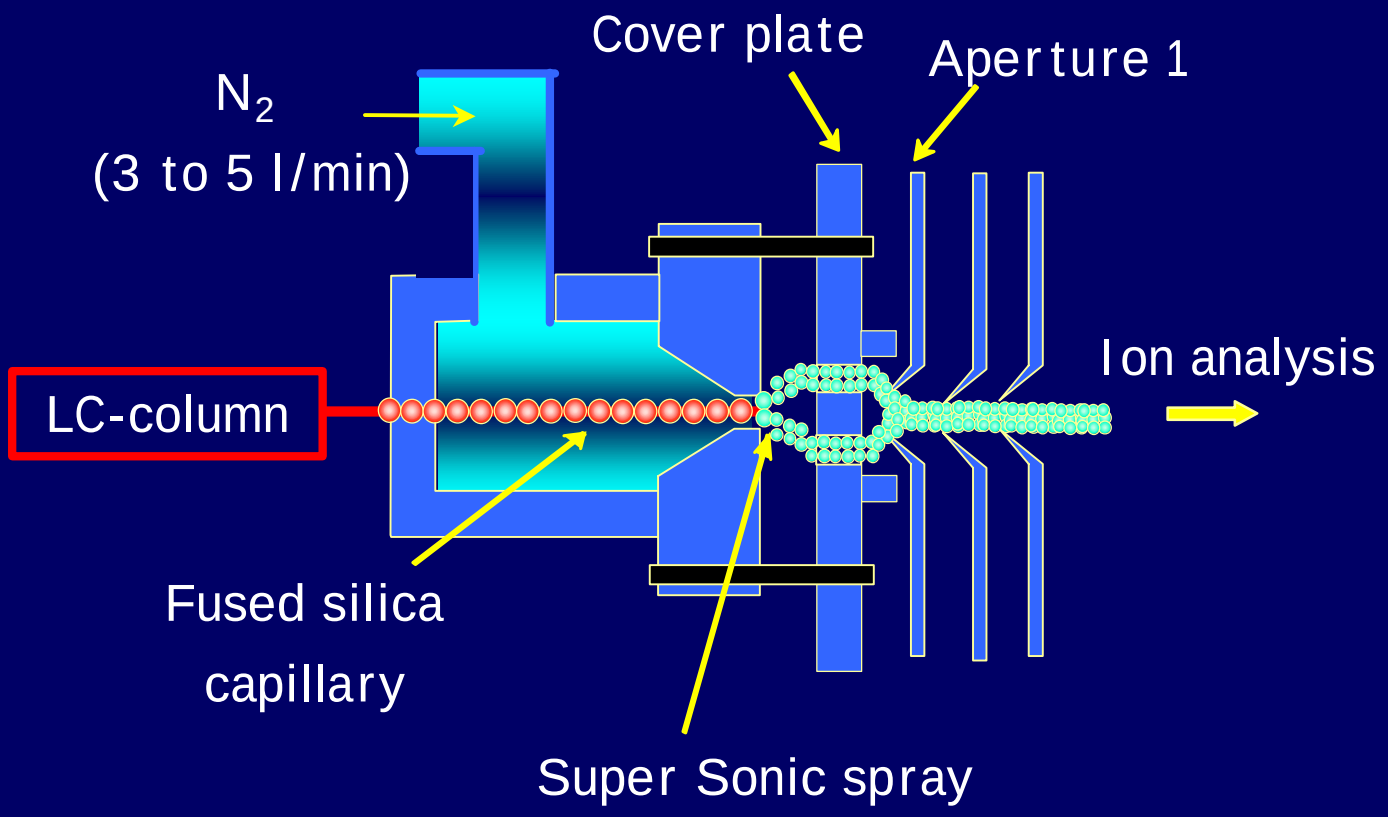
- Coaxial N_2 Flow at sonic velocity (~1 Mach)
- No need for high heating
- No need for electric field

Advantages:

- Thermolabile compounds
- Little in source fragmentation
- Flow rates: 0.1 – 1.0 mL/min.

SSI spectra comparable to ESI spectra

SSI mechanism (M-8000, Merck-Hitachi)



Applications

OPIATES

- Relevant compounds : Heroin, 6-MAM, morph, accod, cod, M-3-G, M-6-G, DHM, DHC
- Interface : Majority APCI and ESI (TSP ’94 & ’96)
- Mass analysis : Q, SIM or QQQ, MRM

Advantages / complications :

- M-3-G and M-6-G can be analyzed
  - Fragmentation into glucuronic acid / aglycon
  - → Time programmed extractor voltage increase

- Large difference in polarity (M-3-G ? heroin)
  - Gradient necessary: Influences ionization process
  - Reproducible reference spectra difficult to obtain

  → Flow programming

- Identification (unequivocal)
  - Molecular ion inadequate

  → CID using LC-MS/MS

NON-CLASSICAL OPIATES : Papaverine, noscapine

- Differentiation non-prescription / prescribed heroin use by urine analysis

AC, C, C-6-G, P, N : Non prescription

AC : very specific

P : short $t_{1/2}$

N : most prevalent, long $t_{1/2}$

- LC-APCI, positive ionization, SIM
  LOD : 0.5-1 ng/mL

HEROIN IMPURITY PROFILING:

AC, C, heroin, 6-MAM, Morph., N, P

- LC-SSI-Ion Trap, positive ionization
- LOD : 2-20 ng/mL
  Less sensitive but sufficient (+scan)
- Monolithic column (Chromolith®)
  Gradient elution : H_2O/AcCN
  Flow : 5 mL/min - split 1/20

COCAINE

- Relevant compounds : cocaine,benzoylecgonine, cocaethylene,… in blood, urine, hair, saliva
- Interface : Majority APCI and ESI (TSP ’96)
- Mass analysis : Q, SIM or QQQ, MRM

Advantages/complications :

- Fragmentation well studied
  → Transition for MS/MS well defined
- Deuterated IS or 2’-methylcoc, 2’-methylBE
- Thermolabile coc-N-oxide can be analyzed
- No artefactual formation of pyrolysis products
- High speed chomatography possible
  → Need for TOF detector instead of scanning instruments

- Analysis of cocaine possible in difficult matrices (Hair, low volumes)

Challenges :

- Method including more coc related compounds
- Identical masses – common fragments
  - coc, norce; BE, norcoc

  → Chrom. separation reproducible t_r
- How to extract them all?
  - Less specific extraction?

  → Ionization suppression?

LSD

- Relevant compounds :
  LSD, nor-LSD, 13-, 14-OH LSD,…
- Interfaces / mass analysis :
  ESI & APCI / Q, SIM; QQQ, MRM

Challenges :

- Low expected levels
- Extensively metabolized
- Photosensitive, thermally unstable
- Adsorption to glass

→ LC-MS(/MS): method of choice

Advantages / complications :

- Fragmentation well known
  new metabolites identified (2-oxo-LSD)

- Often immunoaffinity extraction : saturation problem
- Detection limits : 0.1 and 0.25 ng/mL (LSD, nor-LSD)
- LSD-d_3 as IS has important ion at *m/z* 281 (common with LSD)

→ Monitoring MRM transitions

→ Monitoring other ions (*m/z* 197 and 200)

Conclusion

LC-MS/MS is the method of choice and is already highly optimized

AMPHETAMINE DESIGNER DRUGS

- Relevant compounds :
  Amph., methamph., MDMA, MDEA, MDA, MBDB
- Interface/mass analysis :
  ESI & APCI / Q, SIM, QQQ MRM, Q-TOF

Advantages / complications / challenges :

- Chiral compounds
  Stereospecific differentiation relevant
- Comparative study TSP, ESI, APCI
  TSP most sensitive, requires higher salt conc
  ESI/APCI lower sensitivity
  Variable adduct formation with AcCN

- Detection: Single Q: in-source fragmentation
  Triple Q: more expensive
  Q-TOF: quantitative over 3 decades

CANNABINOIDS

- Relevant compounds :
  THC, THC-COOH, 11-OH-THC, glucuronide

Limited applications :

- Profiling of cannabis samples
  - Info on origin
  - Particle beam interface
- Urine analysis
  - Deconjugation still performed
  - No derivatization for thermolabile THC-COOH

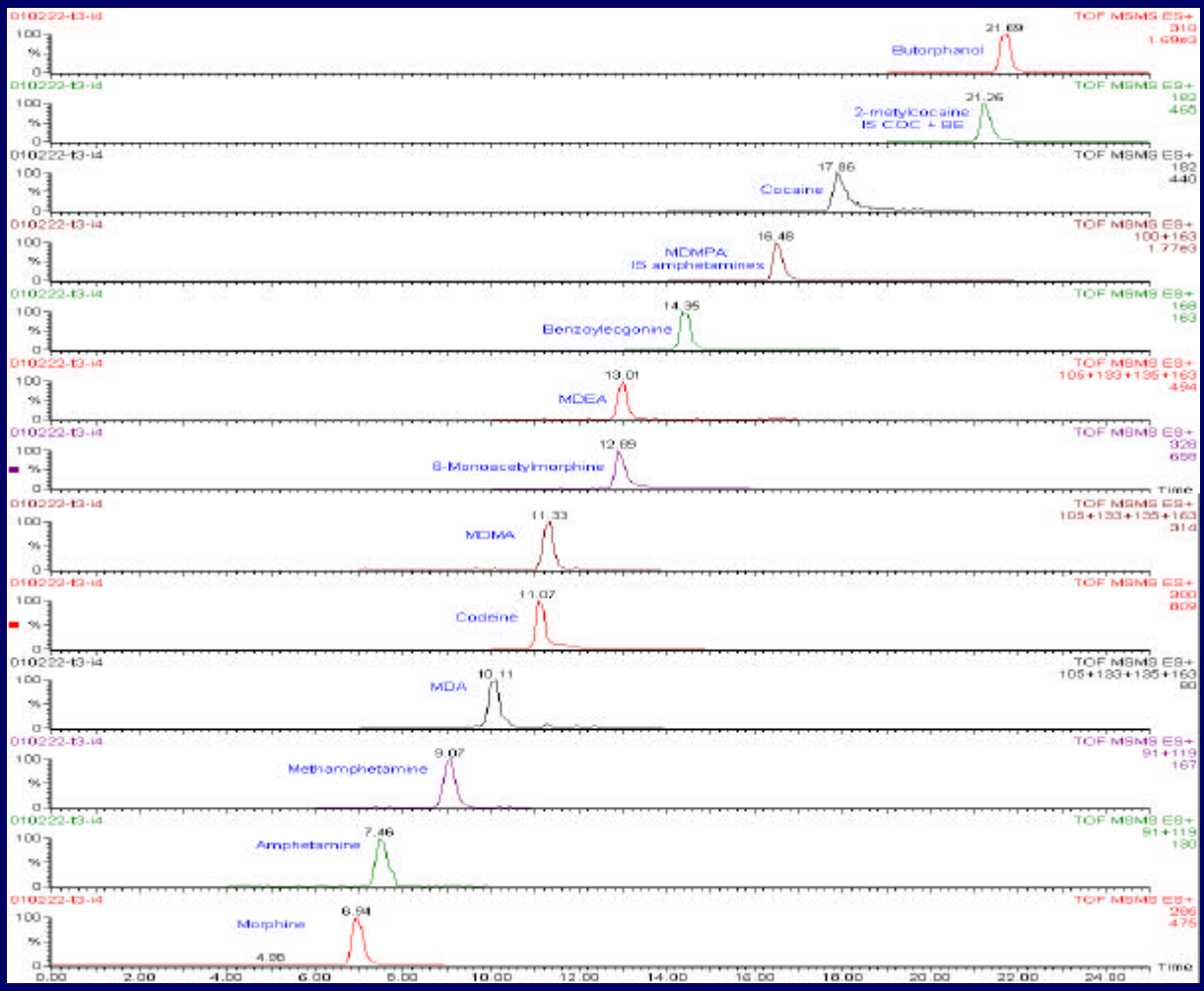
MULTICOMPONENT ANALYSIS

Relevant compounds :
different classes of drugs simultaneously quantified
multidrug use
still target compound analysis

- Drugs of abuse in saliva

All quantified in one run :

Amphetamine	Morphine
Methamphetamine	Codeine
MDA	6-monoacetylmorphine
MDMA (XTC)	Benzoylecgonine
MDEA (EVE)	Cocaine


Butorphanol
21.69
2-metylcocaine
IS COC + BE
21.26
Cocaine
17.86
MDMPA
IS amphetamines
16.48
Benzoylecgonine
14.35
MDEA
13.01
6-Monoacetylmorphine
12.69
MDMA
11.33
Codeine
11.07
MDA
10.11
Methamphetamine
9.07
Amphetamine
7.46
Morphine
6.94
4.86
Time
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00

General conclusions

Beginning

- Toxicologic relevant compounds used for demonstrating LC-MS capabilities

Now

- Ideal (expensive) detector
  - High sample throughput
  - Less efficient separation allowed
    - → Matrix ionization suppression !
  - New possibilities
    - polar metabolites
    - intact glucuronides

- Most applications :
  - Target compound analyses
  - Less suited for profiling purposes

- API interface becomes standard :
  - Persuading hesitating toxicologists
  - Other sources (SSI) specific advantages

Future

- Database of ESI-generated spectra 600/1200 compounds
  - +,-, different fragmentation energy
  - large variability in intensity/fragmentation pattern
  - Standardization needed
- Q-TOF instruments
  - → high identification potential
-

Application shift from target analysis

↓

Screening for unknowns

Cited papers

Other research groups:

Henion, JAT, 1996, 20, 27 (LSD)

Bogusz et al., JAT 2001, 431 (Opiates)

Our research group:

Van Bocxlaer et al. Mass Spectrom. Rev., 2000, 19, 165

Mortier et al., RCM 2001, 15, 1773 (Saliva analysis)

Dams et al., Forensic Sci. Int. 2001, 123, 81 (Impurity profiling)

Dams et al. Anal. Chem. 2002 (accepted for pub.)

Laboratory website:

http://allserv.rug.ac.be/~wlambert