Sonic Spray Ionisation :

Mechanism and application to the LC/MS determination of PMA, XTC and amphetamine in biological samples

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Overview:

- Introduction : LC-MS
- Interfacing
  - Electrospray
  - APCI
- Sonic spray Ionisation
  - Description
  - Mechanism
  - Advantages
- Application to PMA determination
  - Method development
  - Method validation
  - Distribution study
- Conclusions

LC/MS :

Liquid chromatography

- Virtually no derivatization
- Thermolabile compounds
- High Mw/nonvolatile compounds

Mass spectrometry

- Universal detector
- High selectivity
- Sensitive
- Provides info useful for identification of unknowns

⟹ LC-MS powerful tool in (toxicological) bioanalysis

API Interfaces :

- Combine high liquid flow (LC) to vacuum (MS)
- Generate gas phase ions from analytes in solution

Electrospray (ESI)

- Capillary at high voltage (several kV)
- Often pneumatically assisted (ion spray)
- Multiple charging allows protein analysis

Atmospheric Pressure Chemical Ionisation (APCI)

- Uses discharge electrode

Atmospheric Pressure Photo Ionization (APPI)

Sonic Spray Ionisation (SSI)

Sonic Spray Ionisation :

Description :

- Developed and first described by Hirabayashi (94)
- No capillary voltage or heat necesarry
- Sonic gas flow (N_2) coaxial to capillary (3L/min)
- Atmospheric pressure
- Very reproducible ion formation
- Generate multiple charged species (proteins!)

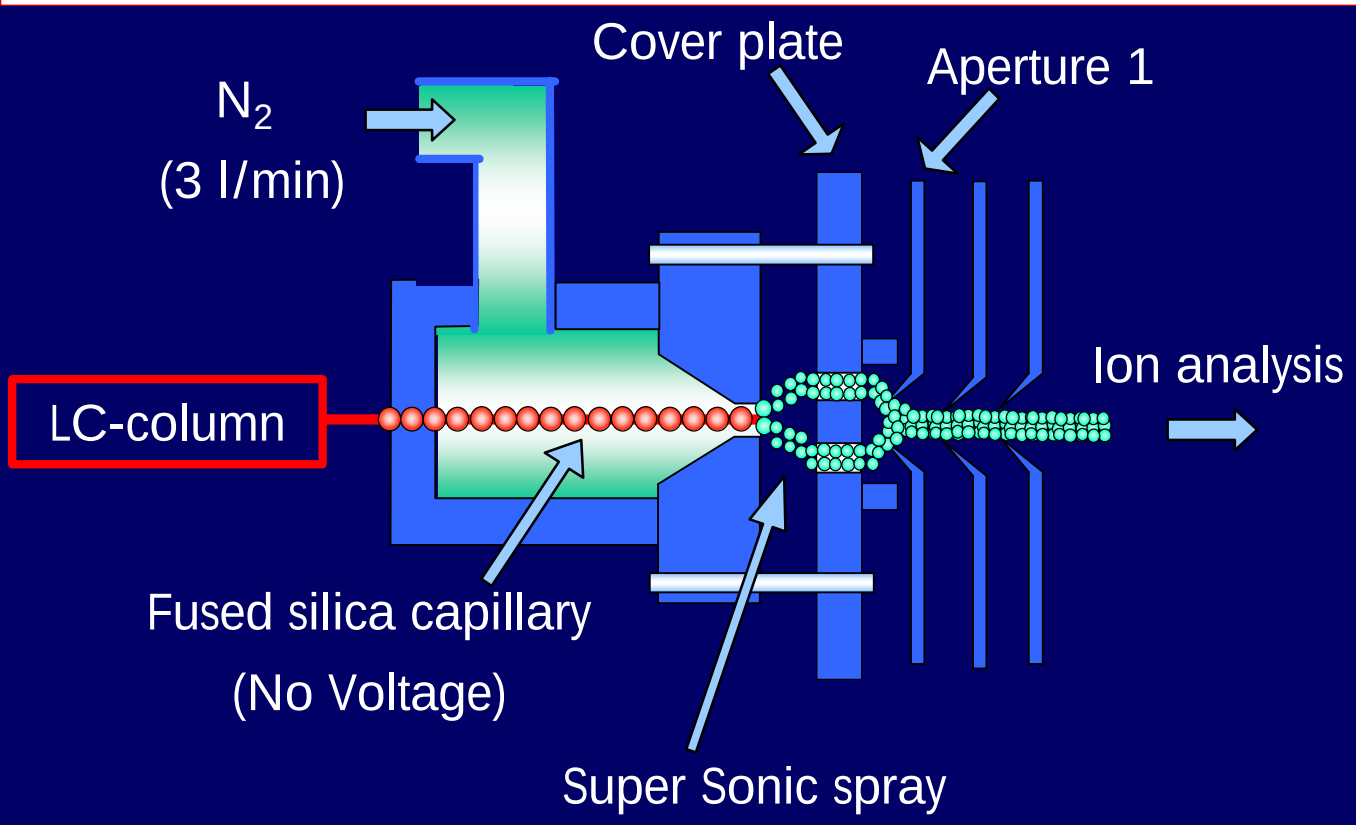
Mechanism according to Hirabayashi :

- Droplets are produced by the shear stress
- Diameter initial droplets 0.5 –5.0 µM (~ESI)
- Near surface of droplet different distribution of positive and negative ions, caused by the surface potential: electrical double layer
- When solution surface undergoes fission: charged droplets are generated

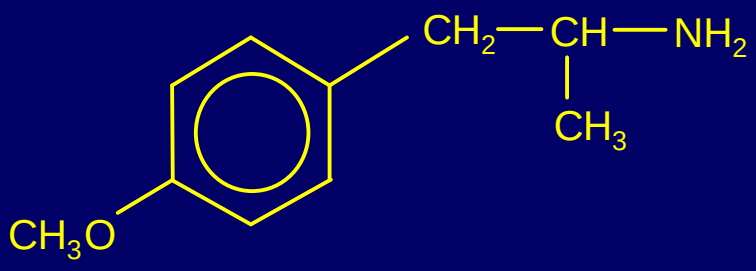
Advantages :

- Allows analysis of thermolabile compounds
- Easy to optimize
- Similar performance to ESI and APCI

M-8000 SSI ion source :



Application to PMA determination :



1. Responsible for several lethal intoxications
2. Structure: amphetamine related
3. Amphetamine alike effect rather than XTC
4. Slow onset, narrow therapeutic index

→ XTC users taking PMA sold as XTC

Effect ? → Overdose !!!

Earlier methodology :

Year	Prep.	Chrom	Detect.	Deriv.
1998	?	GC	MS	?
1998	LLE	GC (dual column)	NPD	PFPA
2001	LLE	GC	MS	HFBA
2001	LLE back	GC (dual column)	NPD	Ac. ac. (anh.)
2001	SPE	GC	MS	MBTFA

1. GC + Derivatization
2. MS preferred: ID and single column
3. Limited info on validation
4. Fatal intoxication involving PMA

⇨ LC-SSI-MS for quantification?

Method development

PMA – MDMA – MDA - amphetamine

IS = ephedrine

1. Sample preparation: LLE

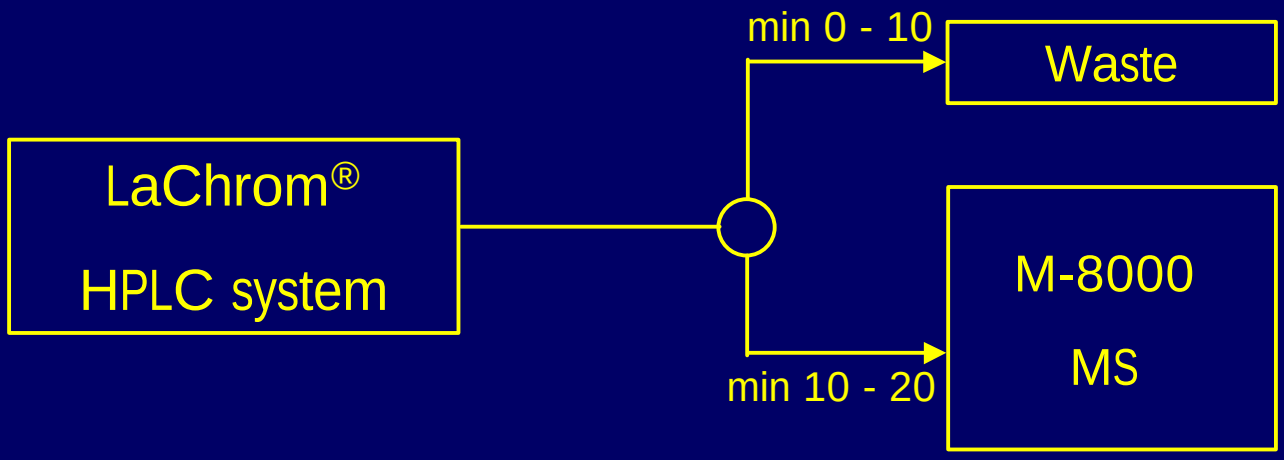
(modified from routine method)

- 0.5 ml blood/urine/tissue* + IS + K_2CO_3
- + 7 ml hexane/ethyl acetate (70/30, v/v)
- Mixing and centrifuging
- Evaporation of organic layer (+ acid alcohol)
- Redissolved in 100 µl eluent
- 20 µl injected on column

* 1 ml, ¼ diluted with water

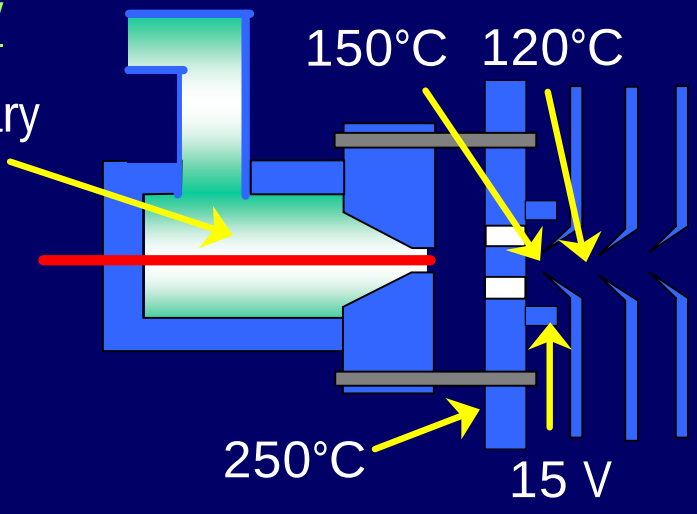
2. Chromatography

Column: phenyl column, 100 x 2.1 mm, 3 µm
Flow: 0.3 ml/min, no splitting
Mobile phase: Formic acid (0.001%) / AcCN
Gradient: from 6 to 50% AcCN
Switch box: First 10 min directed to waste



3. Mass spectrometry

SSI:



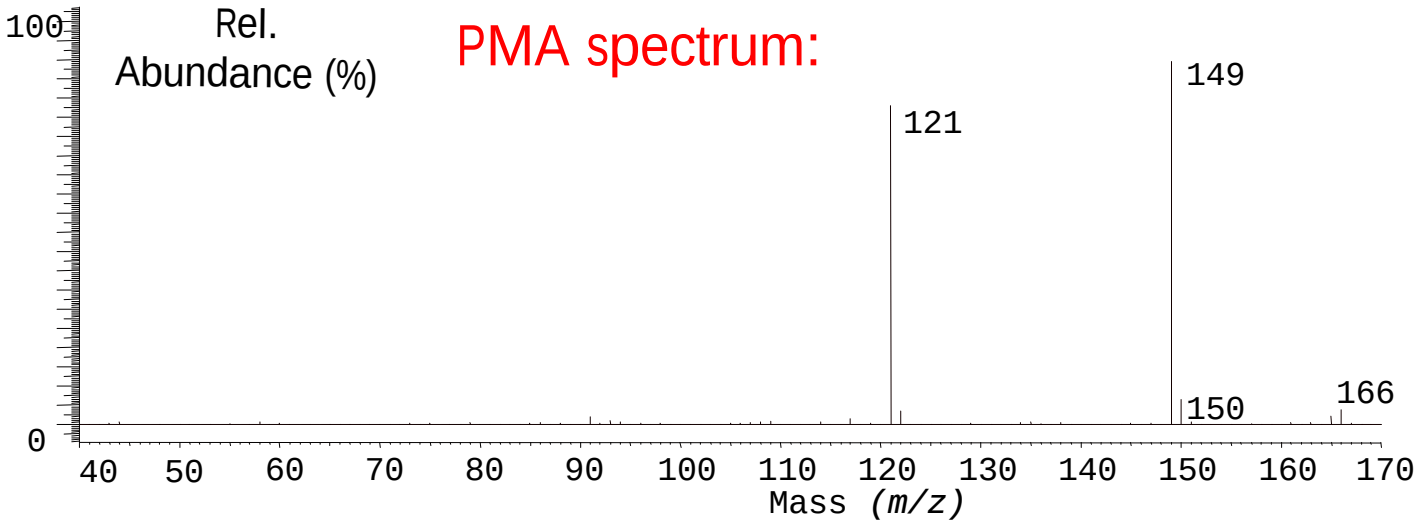
Ion trap MS:

- *m/z* 50 – 200 accumulated
- Protonated analytes isolated and fragmented
- Helium: buffer gas

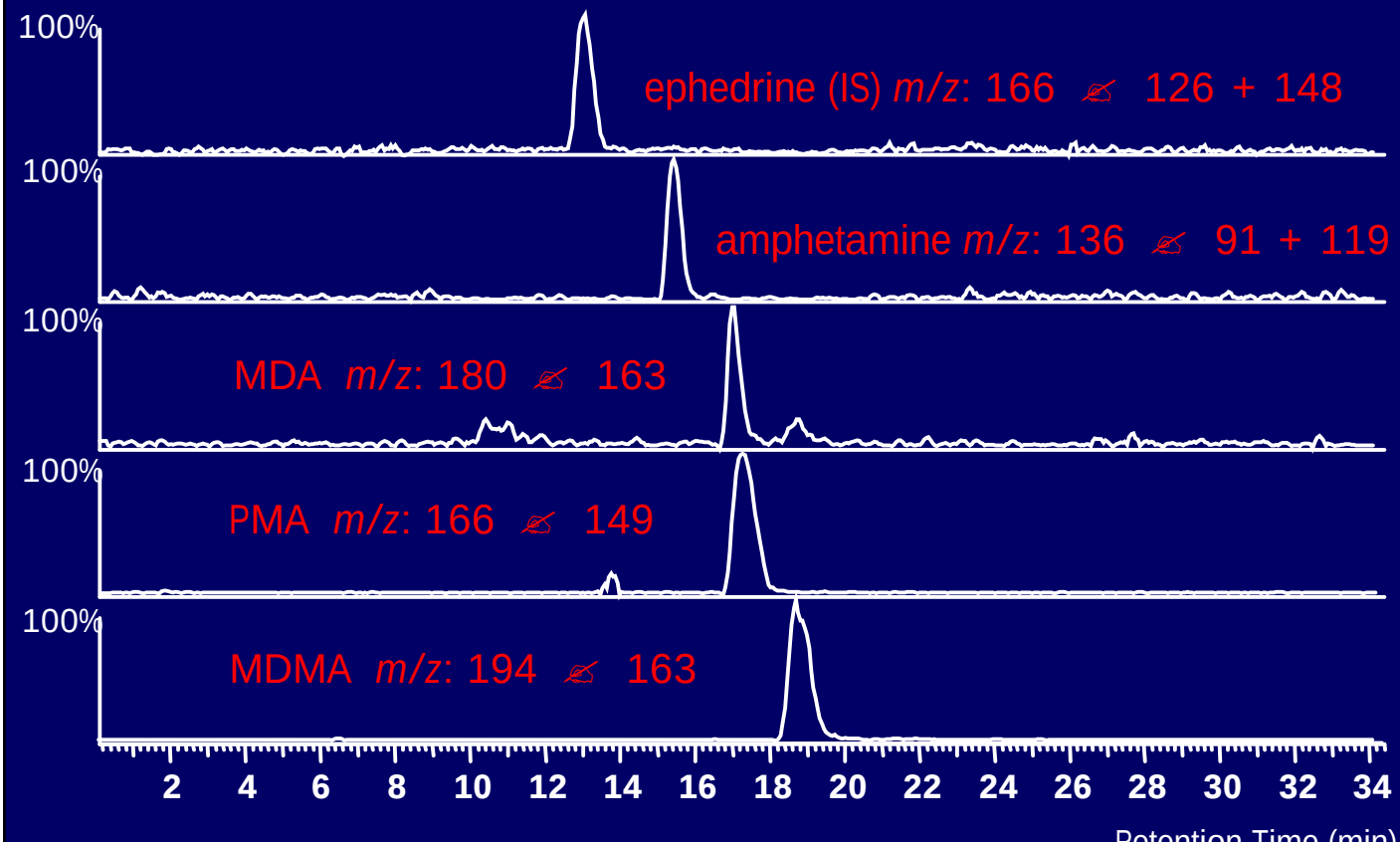
MS²:

Analyte	Precursor *(m/z)*	Product *(m/z)*
Ephedrine (IS)	166.1	126 + 148
Amphetamine	136.1	91 + 119
MDA	180.1	163
PMA	166.1	149
MDMA	194.1	163

PMA spectrum:



4. Chromatogram (real urine sample):



Method validation (at low and high conc)

n = 3	Blood	Urine	Tissue
LOD	2.5 –5 ng/ml	2.5 –5 ng/ml	5 –10 ng/g
LOQ	10 ng/ml	10 ng/ml	20 ng/g
Recovery	83 –103%	86 –104%	93 –107%
Accuracy	< 12%	< 14%	< 17%
Precision WD	< 15 RSD	< 16 RSD	< 14 RSD
Precision BD	< 18 RSD	< 16 RSD	< 15 RSD

Calibration curves: 10 –1000 ng/ml blood/urine
20 –2000 ng/g tissue

Intoxication with amphetamine drugs of abuse

1. Establish cause of death
    - PMA identified by LC/MS: new method developed
    - Toxic levels in femoral blood (µg/ml):

	PMA	MDMA	MDA	Amph.
Results	1.6	1.1	0.4	0.2
Lethal c	>0.5?	>1?	?	>0.5?

Hyperthermia , disseminated intravascular coagulation as cause of death.

Distribution study

- Analysis of various biological matrices (often analytical challenge) Blood, urine, lung, liver, kidney, muscle, brain, stomach content, bile, adipose tissue

⇨ for lipophyllc matrices: LLE back extraction

- Establish distribution and postmortem distribution
  - Elevated blood levels in center compared to periphery proove redistribution
  - High lung concentrations
  - High brain concentrations (up to 4 µg/g PMA)
  - Iliopsoas muscle good alternative to blood
- Interesting for future intoxications

Conclusions :

- SSI successfully adopted, even for complex matrices
- LLE: good recovery and reproducibility
- sensitive, reliable
- applied to real urine, blood and tissue samples

Acknowledgements and cited literature :

- Prof. S. Van Calenbergh, Dr. E. De Letter, Prof. M. Piette, G. Van Nuffel, Merck KGaA, Darmstadt.

- PMA method : Mortier et al. RCM, 865 (2002)
  SSI : Hirabayashi et al. Anal. Chem., 4557 (1994)
  SSI : Hirabayashi et al. Anal. Chem., 2878 (1995)

- This presentation is on the laboratory website:
  http://allserv.rug.ac.be/~wlambert

- Thank you for your attention!!!