A validated method for the determination of PMA and other amphetamine-related designer drugs in biological matrices by liquid chromatography sonic spray ionization mass spectrometry

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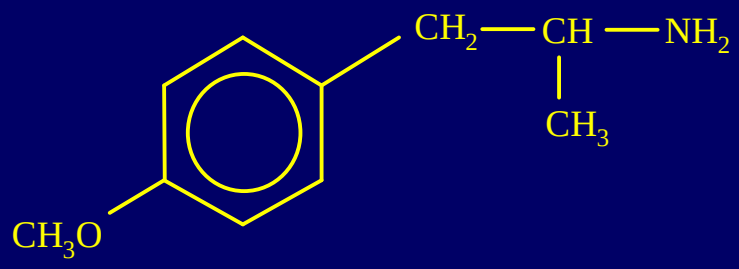
Paramethoxyamphetamine:



1. Structure: amphetamine related
2. Amphetamine alike effect rather than XTC
3. slow onset, narrow therapeutic index

→ XTC users taking PMA sold as XTC

Effect? → Overdose

Previous 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

LC/MS:

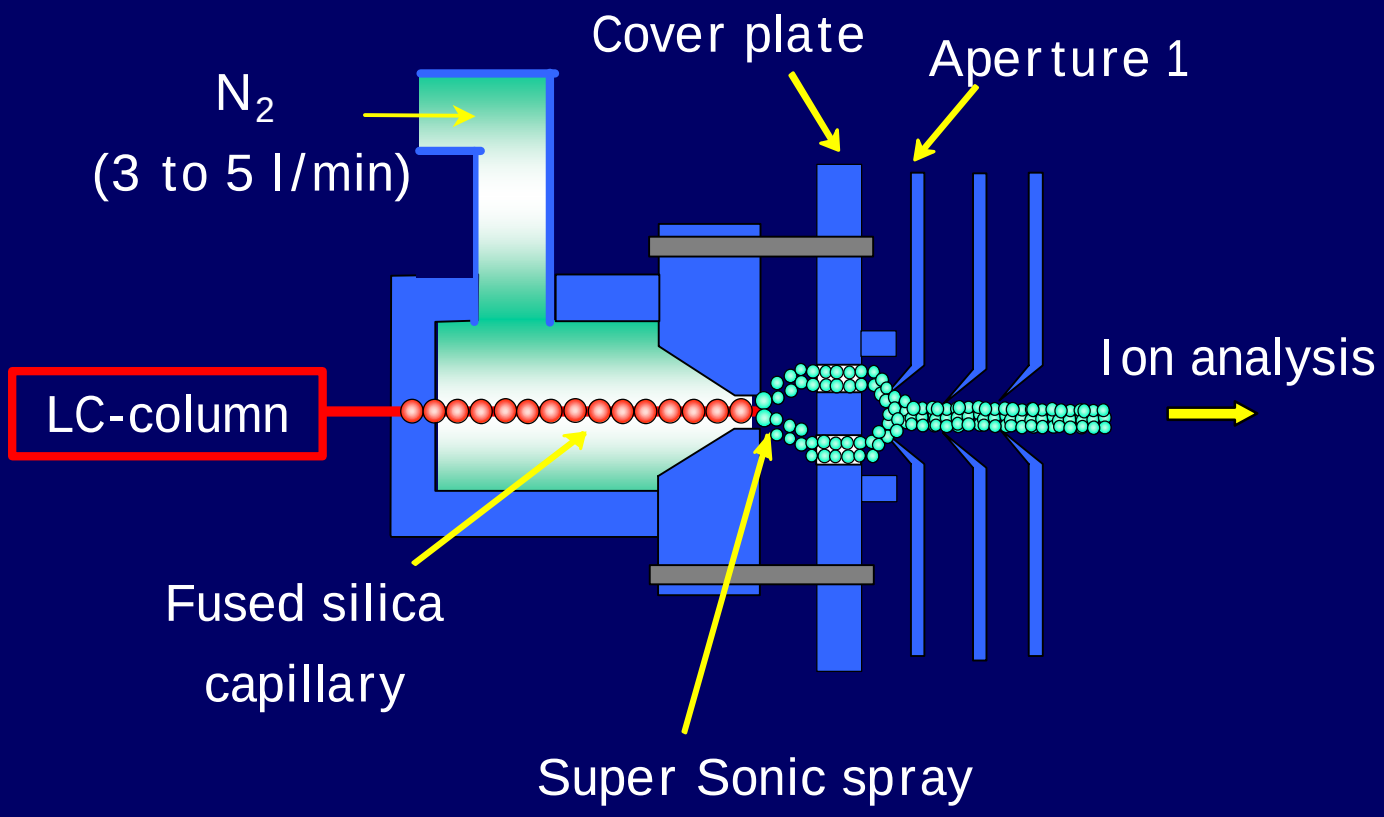
- Sensitive
- No derivatization
- Identification reliable by MS^2

→ ION TRAP MS^2 (M-8000 Merck-Hitachi)

LC/MS interface:

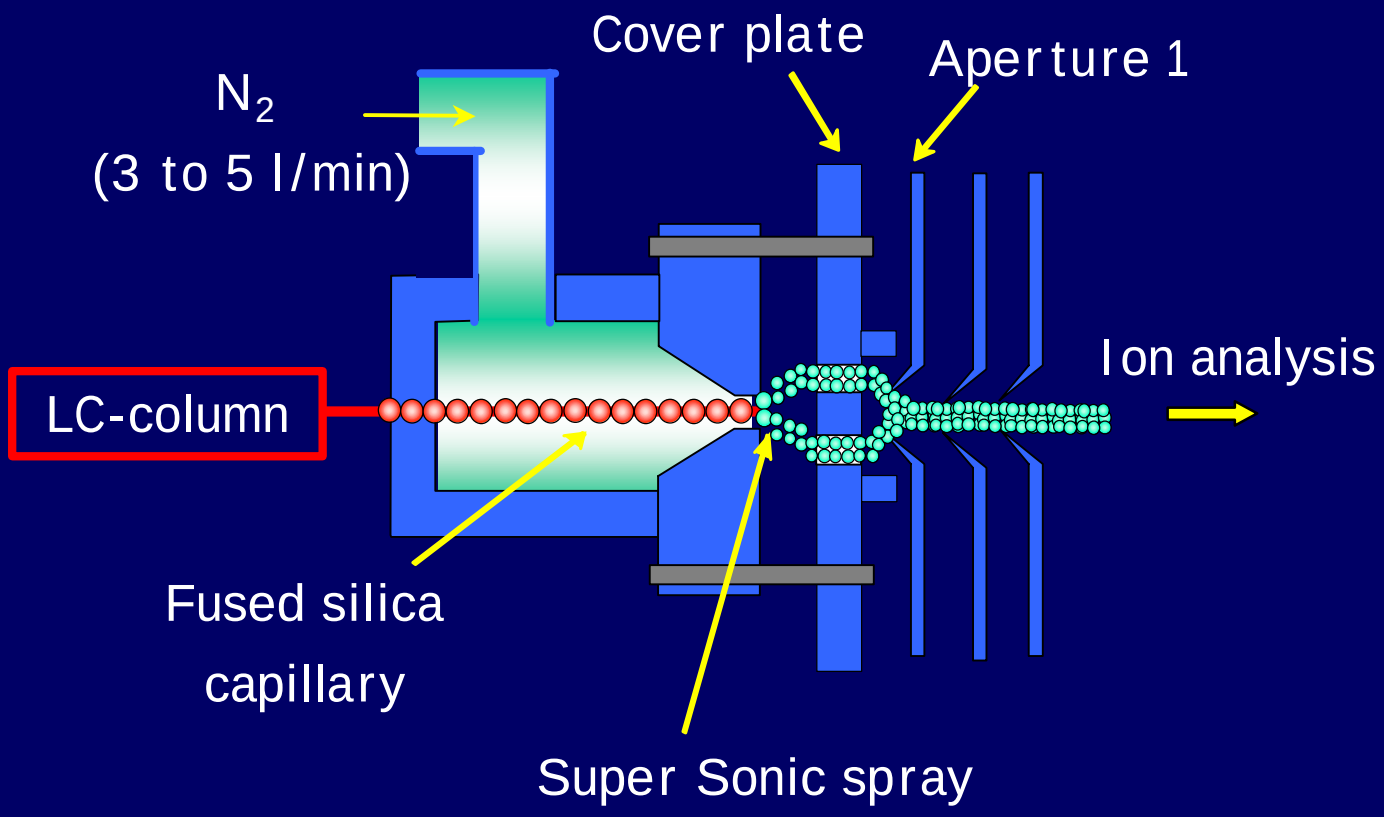
- APCI/ESI ⟷ SSI (sonic spray ionization)
- novel API source
- easy to optimize

M-8000 SSI ion source



(With permission adapted from Tom Benijts)

M-8000 SSI ion source



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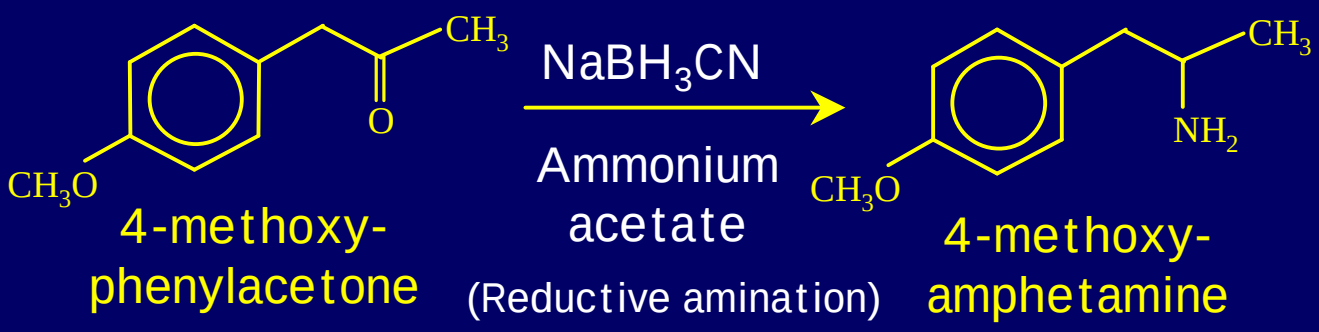
PMA synthesis



4-methoxy-phenylacetone

4-methoxy-amphetamine

- Fast, easy and cheap (one step via Shiffs' base)
- LLE for purification
- confirmed by NMR and MS (GC and LC)

Method development

PMA – MDMA – MDA - amphetamine

IS = ephedrine

1. Sample preparation: LLE

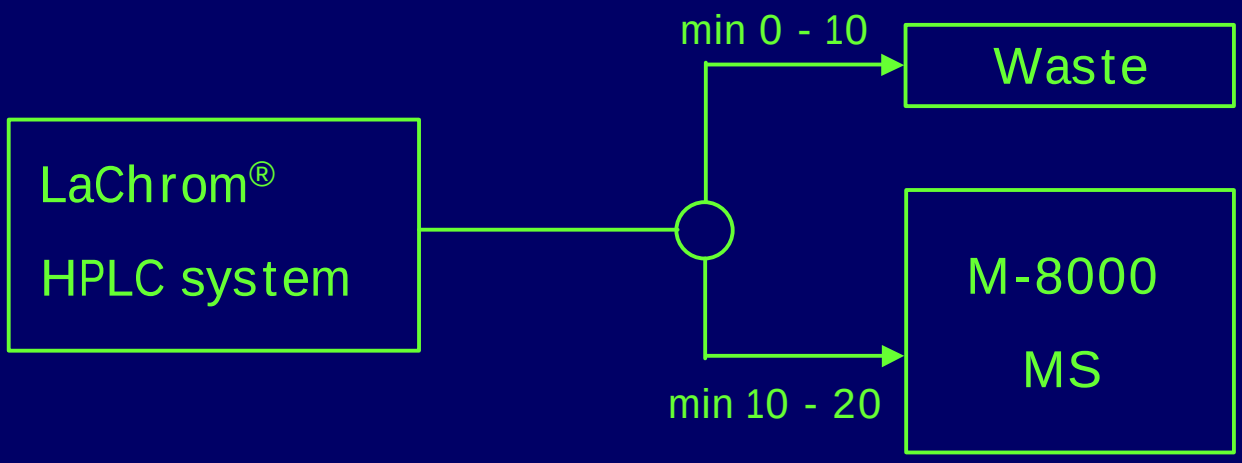
(modified from routine)

- 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

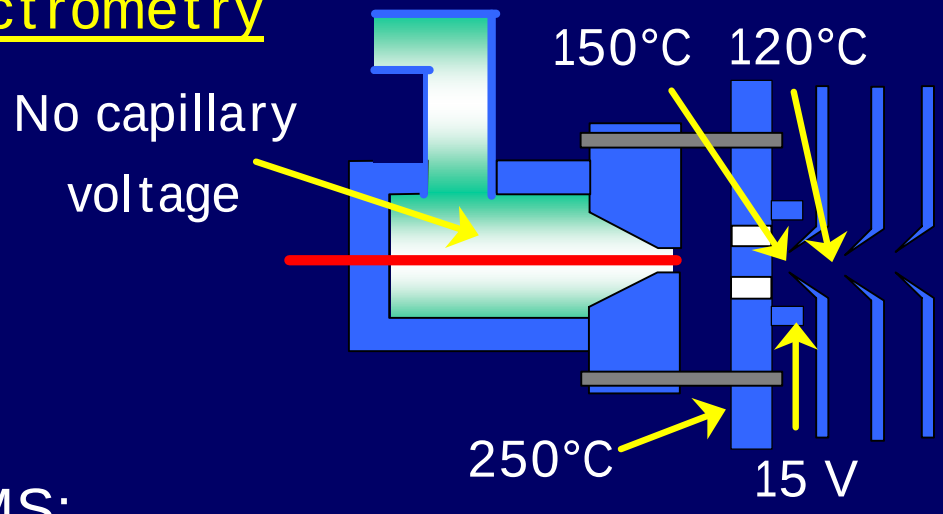
2. Chromatography

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:



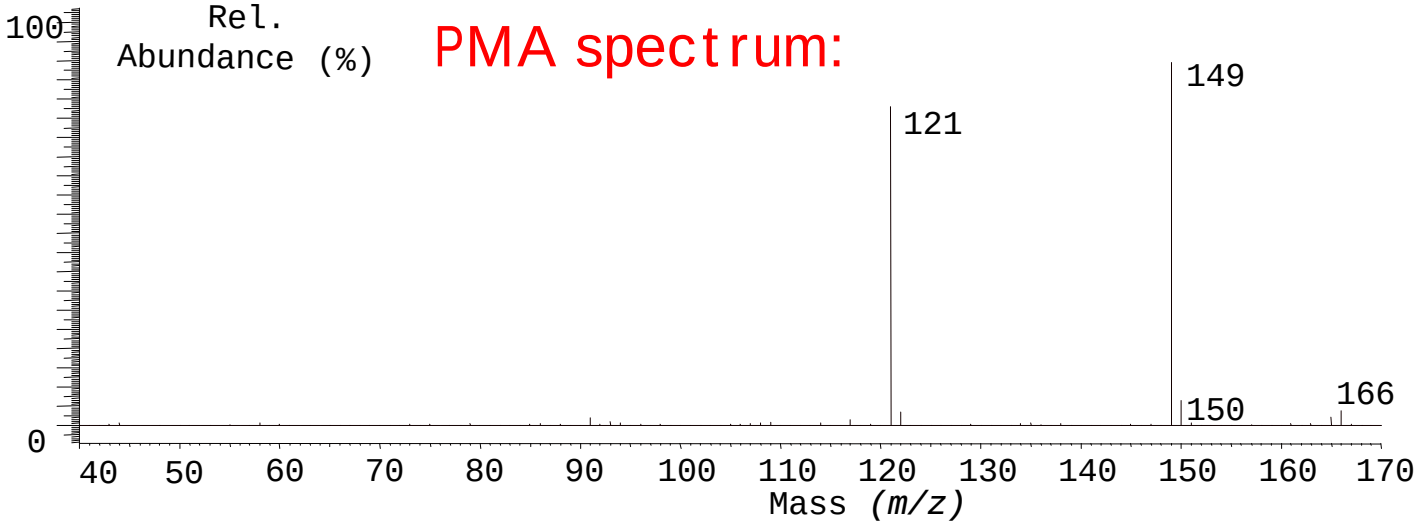
Ion trap MS:

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

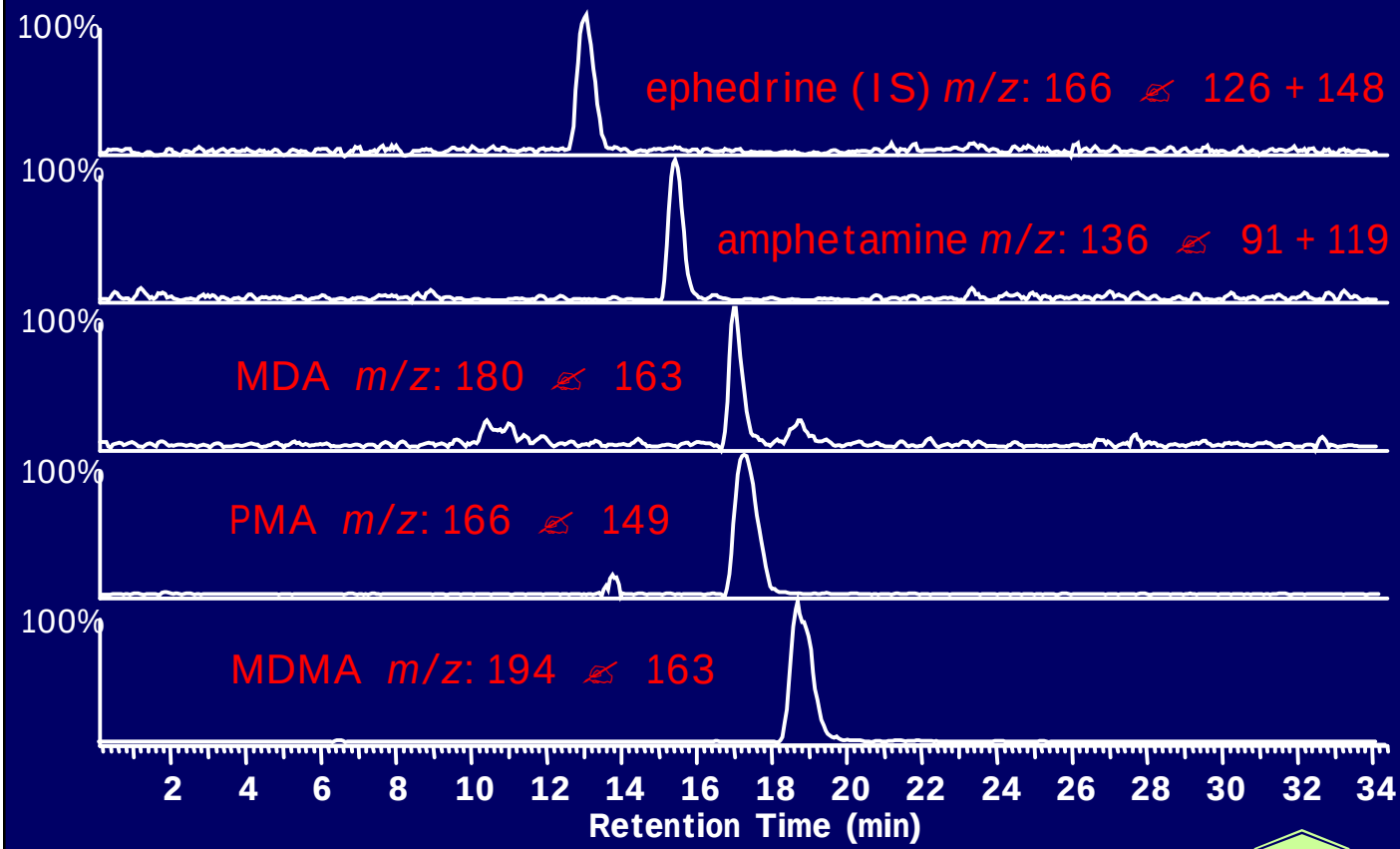
MS^2:

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):



Validation (at low and high °C)

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

Conclusion

- SSI successfully adopted
- LLE: good recovery and reproducibility
- sensitive, reliable
- applied to real urine, blood and can be applied to tissue samples

Accepted for publication in Rapid Communications in Mass spectrometry'

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