Simultaneous determination of eight drugs of abuse and codeine in saliva by liquid chromatography tandem mass spectrometry

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Why saliva ?

1. Easy to sample
   - Almost always available (? urine)
   - No medical personnel needed (? blood)
   - Painless, safer (? blood)
   - Difficult to adulterate (? urine)
2. Saliva concentrations comparable to plasma ?
   - Lipophilic basic drugs often ? C.
   - Contamination ??
3. Relatively clean matrix
   - Few lipids

Measuring drugs of abuse in saliva

1. Often GC/MS
   - Derivatization
   - Extensive sample clean-up required
2. Often one group of substances
   - Amphetamines
   - Opiates
   - Cocaine and benzoylecgonine
3. LC/MS seldom reported

Why LC/ESI/MS QTOF?

1. LC/MS
   - Simple sample prep
   - No derivatization
2. Quadrupole Time of Flight
   - Amphetamines
   - Opiates
   - Cocaine and benzoylecgonine

   } In one run
3. Reliable identification
   - Full spectrum without loss in sensitivity
   - Accurate mass measurement

Sample preparation (I)

1. Protein precipitation: easy and fast
   - Suffers from matrix suppression (ESI)
     - → better sample clean-up required
     - → labelled internal standards ?
     - → APCI (sensitivity?)
2. Mixed mode solid phase extraction
   - Ideally suited for multiple drug extraction

Sample preparation (II)

Bond Elut Certify

200 µl saliva + IS (+ standard) + phosphate buffer

Conditioning : 3 ml methanol

2 ml phosphate buffer pH 6.0

Loading sample : 2 ml/min

Wash : 2 ml acetic acid (0.1M fast)

: 2 ml methanol (fast)

Dry : 5 min

Elute : i-PrOH/CH_2Cl_2/NH_4OH

Evaporate + HCl and redissolve in 200 µl eluent

Chromatography

Injection volume: 50 µl

Column: phenyl column, 100 x 2.1 mm, 3 µm

Flow: 0.2 ml/min, no splitting

Mobile phase: NH_4Fo, 10 mM, pH 5.0

Gradient: from 6 to 67.6% MeOH

Analysis time: 34 min.

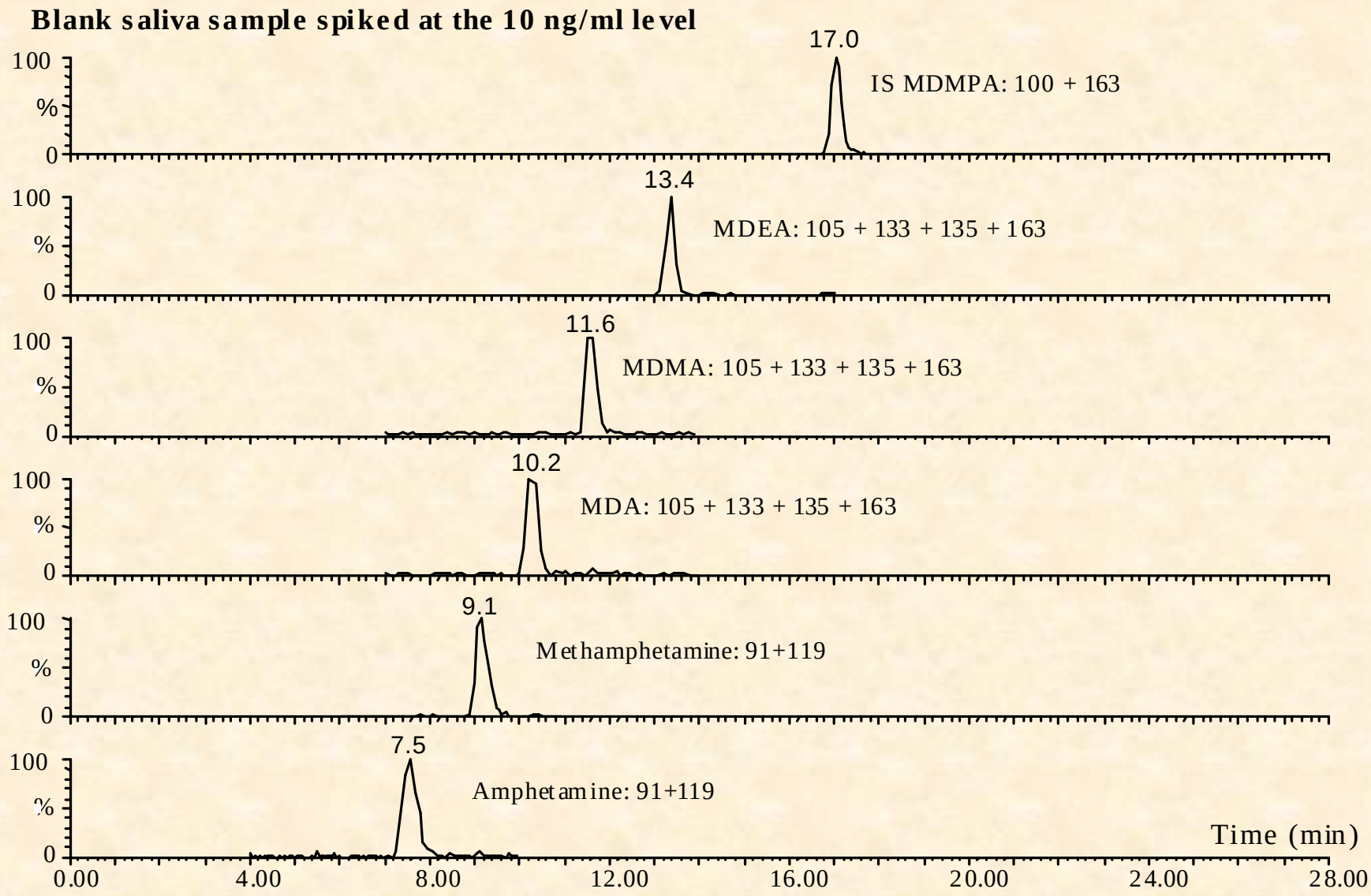
Internal standards:

- Opiates : Butorphanol
- Amphetamines : MDMPA
- Cocaine (and BE) : 2 ’-methylcocaine

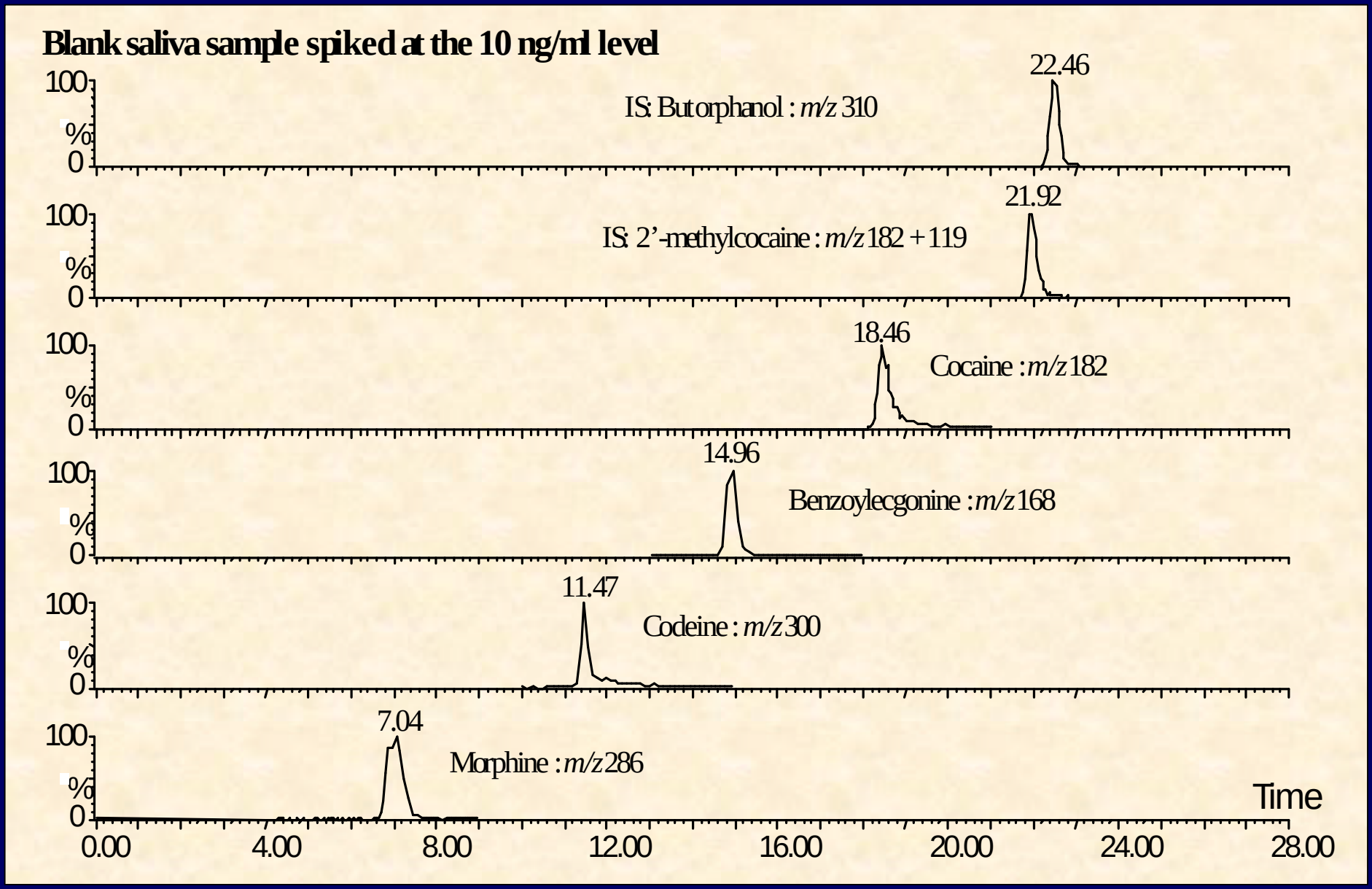
Mass spectrometry

Compound	Fragmentation	Cone V.	Coll. En
MDMPA	236 ✍ 100 + 163	15	19
Butorphanol	328 ✍ 310	37	32
2 ’-methylcocaine	318 ✍ 182 + 119	37	28
Amphetamine	136 ✍ 91 + 119	15	14
Methamphetamine	150 ✍ 91 + 119	15	18
MDA	180 ✍ 105 + 133 + 135 + 163	15	16
MDMA	194 ✍ 105 + 133 + 135 + 163	15	16
MDEA	194 ✍ 105 + 133 + 135 + 163	15	18
Morphine	286 ✍ 286	38	13
Codeine	300 ✍ 300	38	13
Benzoylecgonine	290 ✍ 168	37	27
Cocaine	304 ✍ 182	37	26

Chromatogram I: Amphetamines



Chromatogram II: Opiates and cocaine



Validation (at low and high C)

Extraction recovery: Morphine 58 – 64%
Others: > 74%

No Matrix suppression: (5 diff. individuals)

Precision: Within and between day: < 16.8%

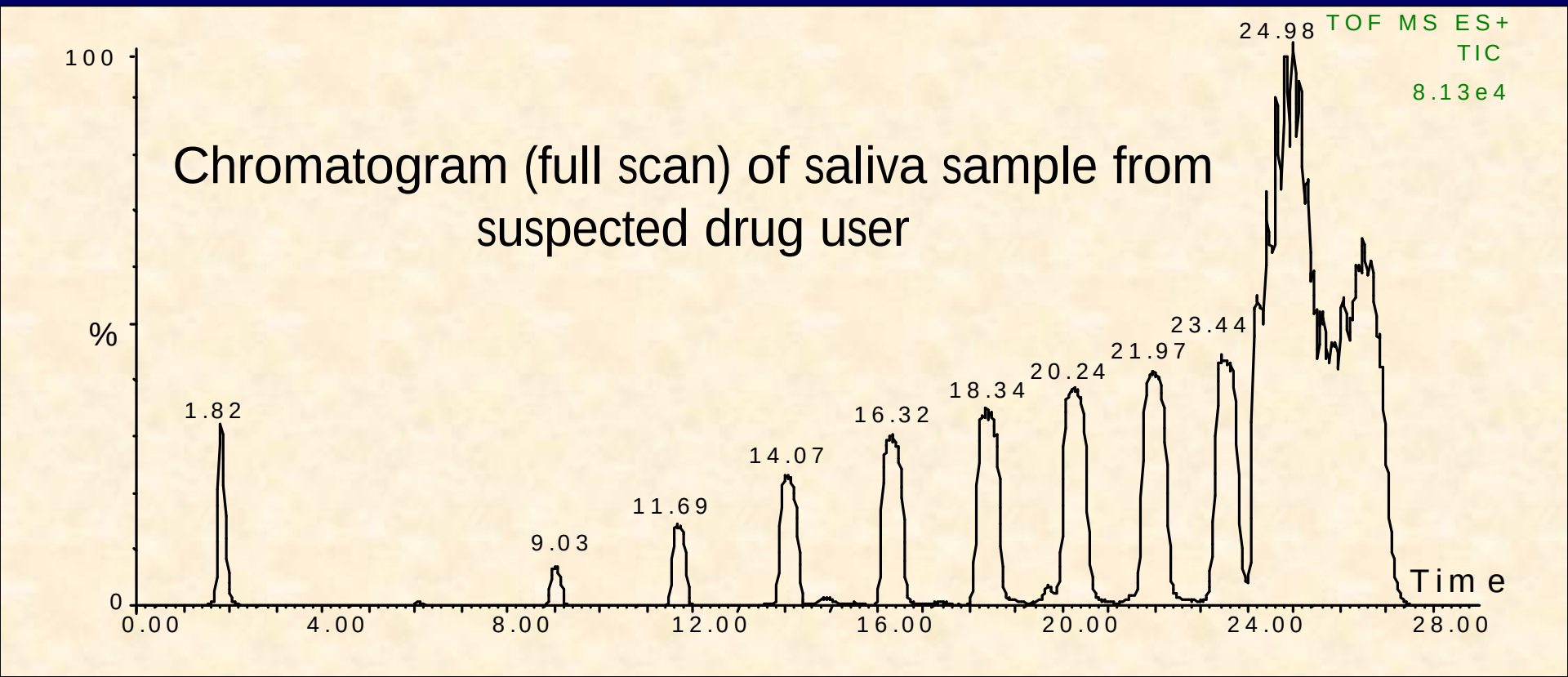
Accuracy: < 12%

LOQ: 2 ng/ml saliva

Calibration: Quadratic curves 2 – 100 ng/ml

Samples

- 41 saliva samples from suspected drug users
- Samples: peak area of IS irreproducible
- Calibrators: peak area of IS reproducible



Contamination origin

- Contamination from polymer releasing combinations of monomers
- Origin: Sample device (HSW Henke –Sass, WOLF GMBH)
- Suppression of later eluting compounds (IS)

Conclusion

- Method validated for the determination of drugs of abuse in saliva, obtained by spitting

- Sampling device should be taken into account when developing analytical procedures

Acknowledgements

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