

Attenuated Total Reflection Spectroscopy on a One-Step-Extract from Saliva utilized for Cocaine Detection

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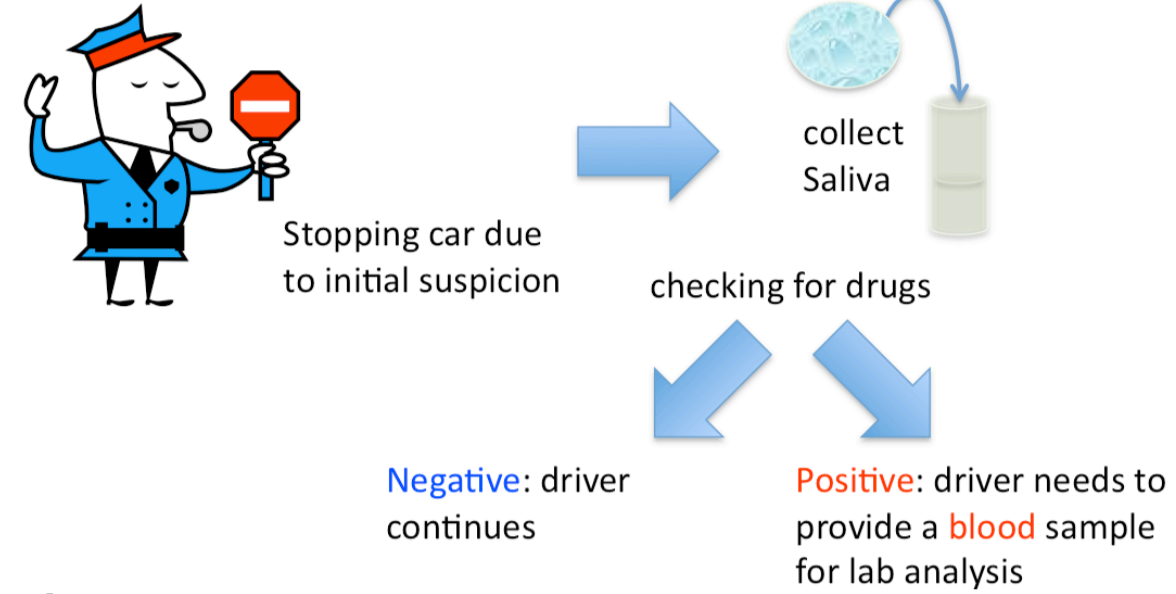
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Introduction, goals, challenges and solutions

Lab-on-a-chip-sensors are powerful tools in diagnostics, e.g., detecting drugs in body fluids, due to their low costs and quick results. Saliva serves as a better matrix than blood or urine because it can be collected non-invasively and by less trained staff. Up to now there is a lack of easy-to-use quantitative methods.

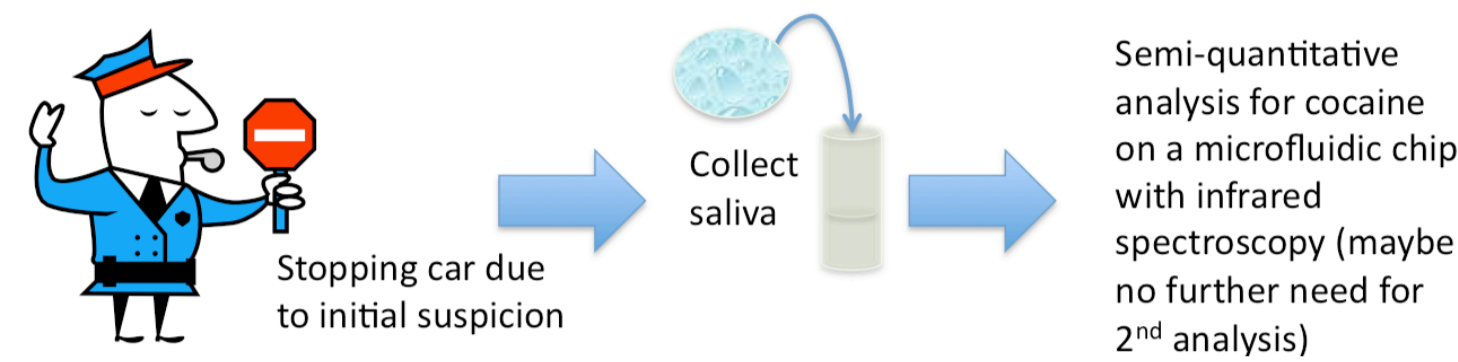
Current Situation:



Problems:

- Relative high false positive/negative results [1]
- No quantitative result on the street (risk assessment)
- Second expensive test analysis necessary

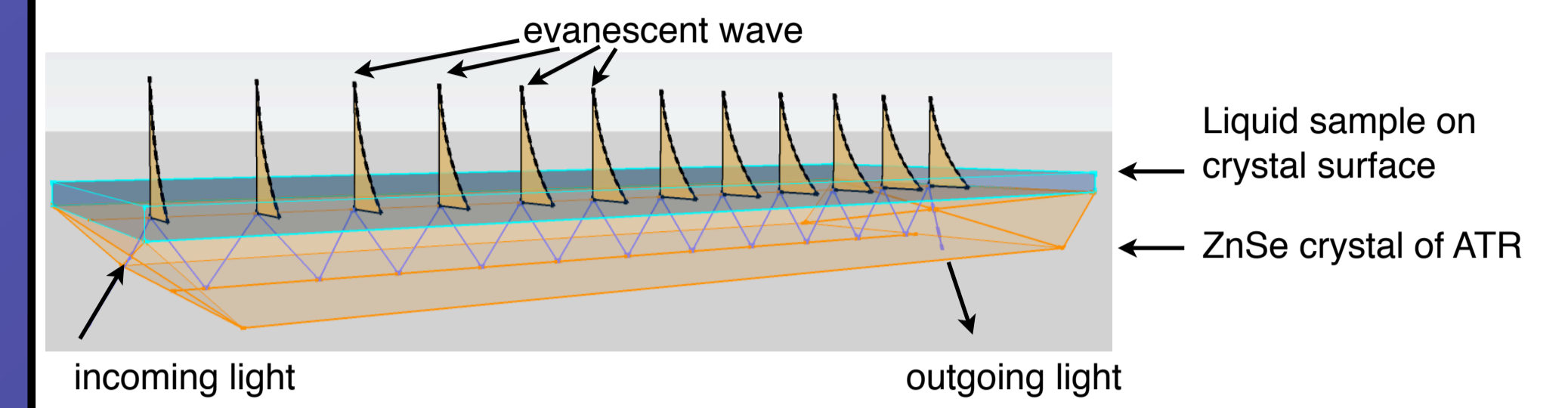
Future perspective with industrial partner:



Challenges:

- High water and water vapour absorption → Drug extraction on the enclosed chip
- Interfering substances → Extensive prestudies [2]
- High sensitivity needed for determining cocaine & metabolites → Lower limit of detection and potential preconcentration

Method: Attenuated Total Reflection (ATR) spectroscopy

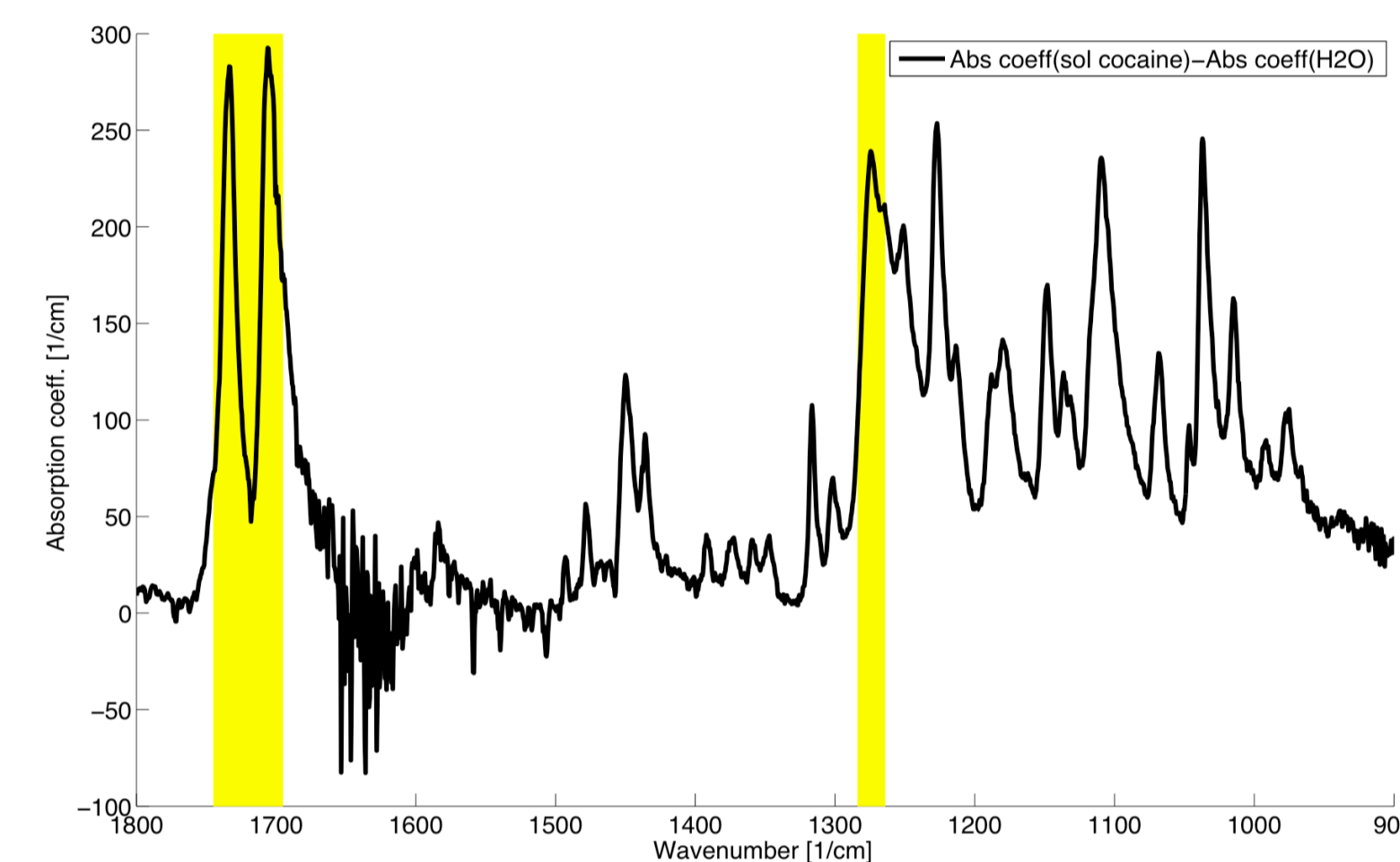
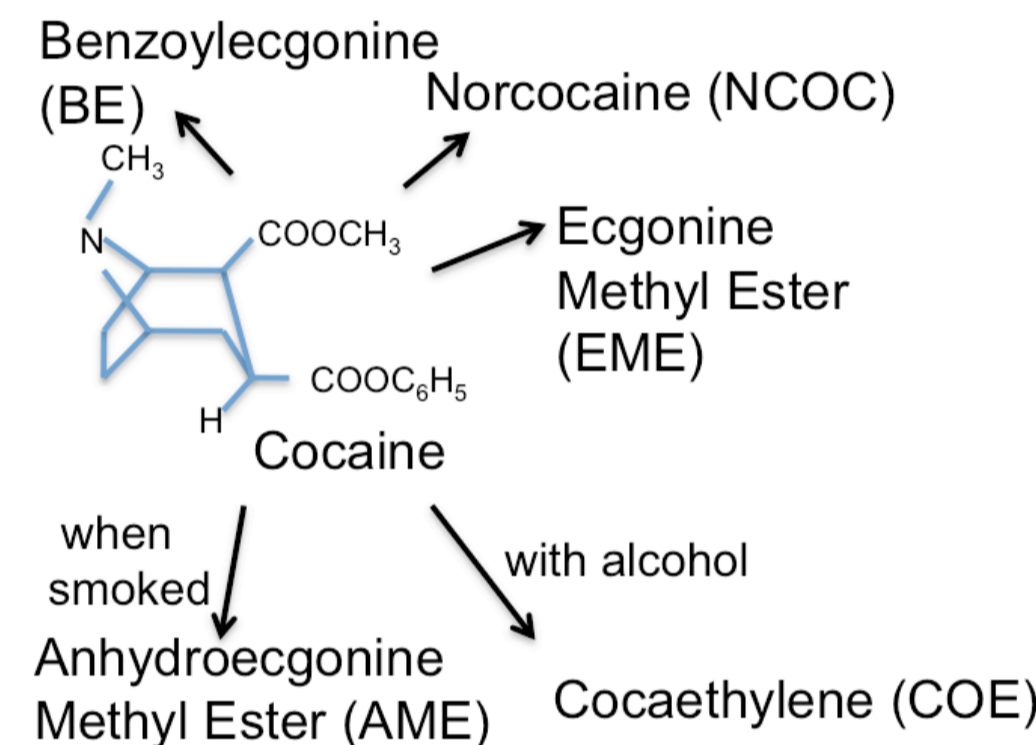


- ATR spectroscopy is selected as promising method for absorption measurements in liquids
- Broadband studies were performed with an FTIR Spectrometer (Paragon 1000 PC) equipped with an ATR unit
- In the ATR unit the light is reflected eleven to twelve times in a ZnSe crystal creating an evanescent field. This field is penetrating the sample. In consequence a decrease in intensity of the outgoing light is correlated with absorption of the sample.
- Measurements within selected narrow spectral ranges are currently performed with QCLs

Challenge of low detection limits

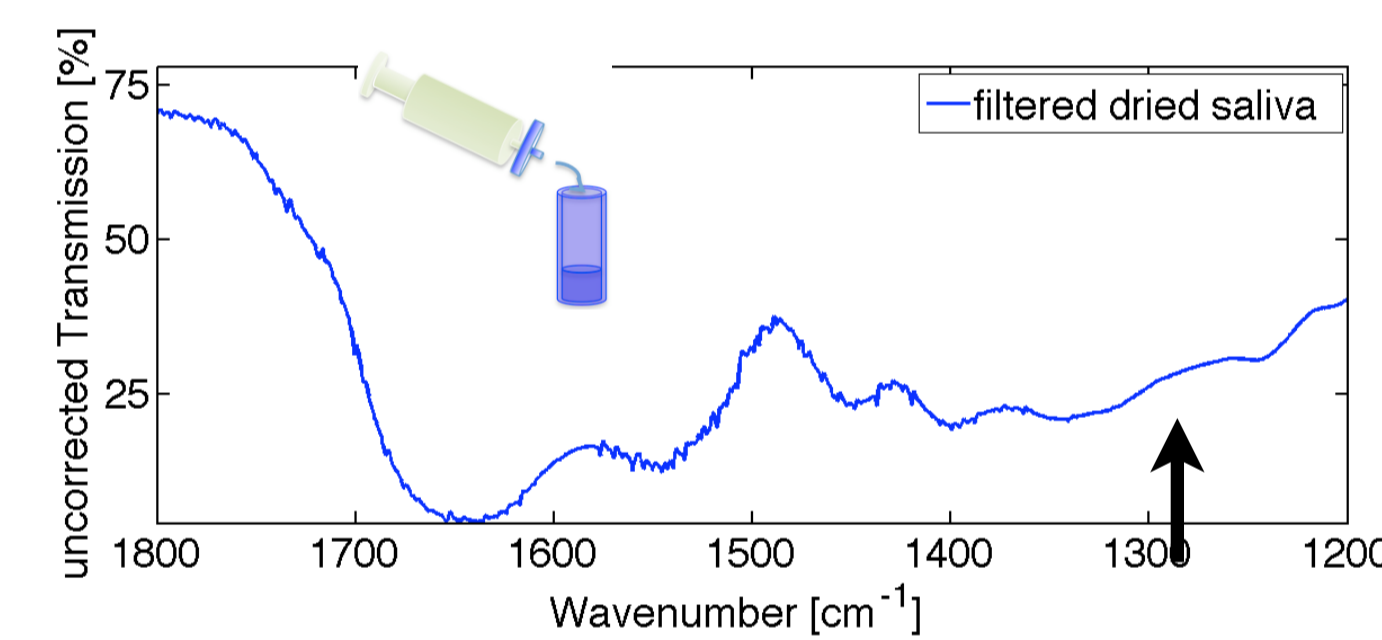
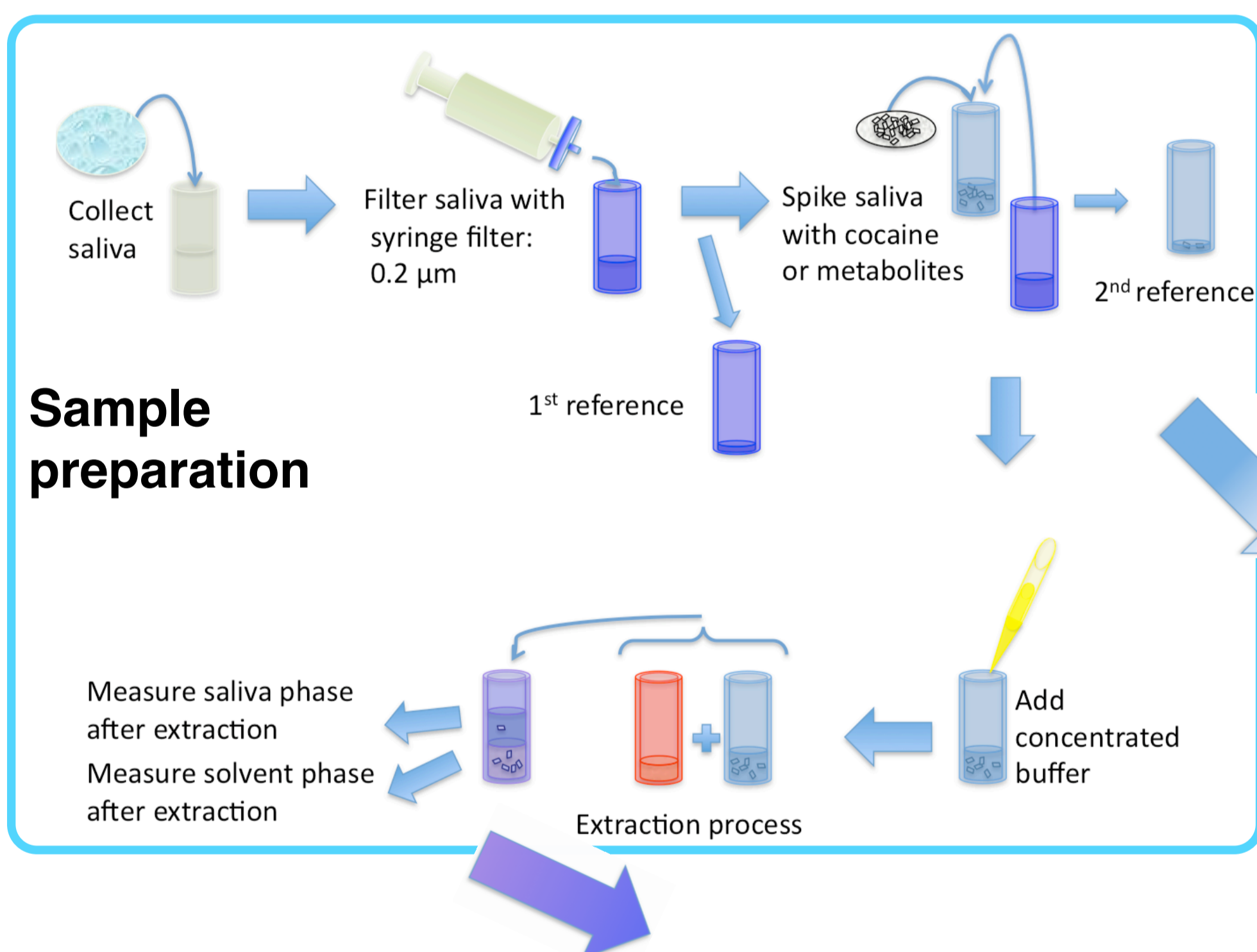
- Cocaine concentration in saliva is up to 500 µg/ml [3]
- BE concentration is up to 3 µg/ml [4]
- EME concentration up to 0.2 µg/ml [5]
- NCOC concentration up to 0.1 µg/ml [3]
- AME concentration up to 4 µg/ml [3]

Metabolites of cocaine according to [6]:

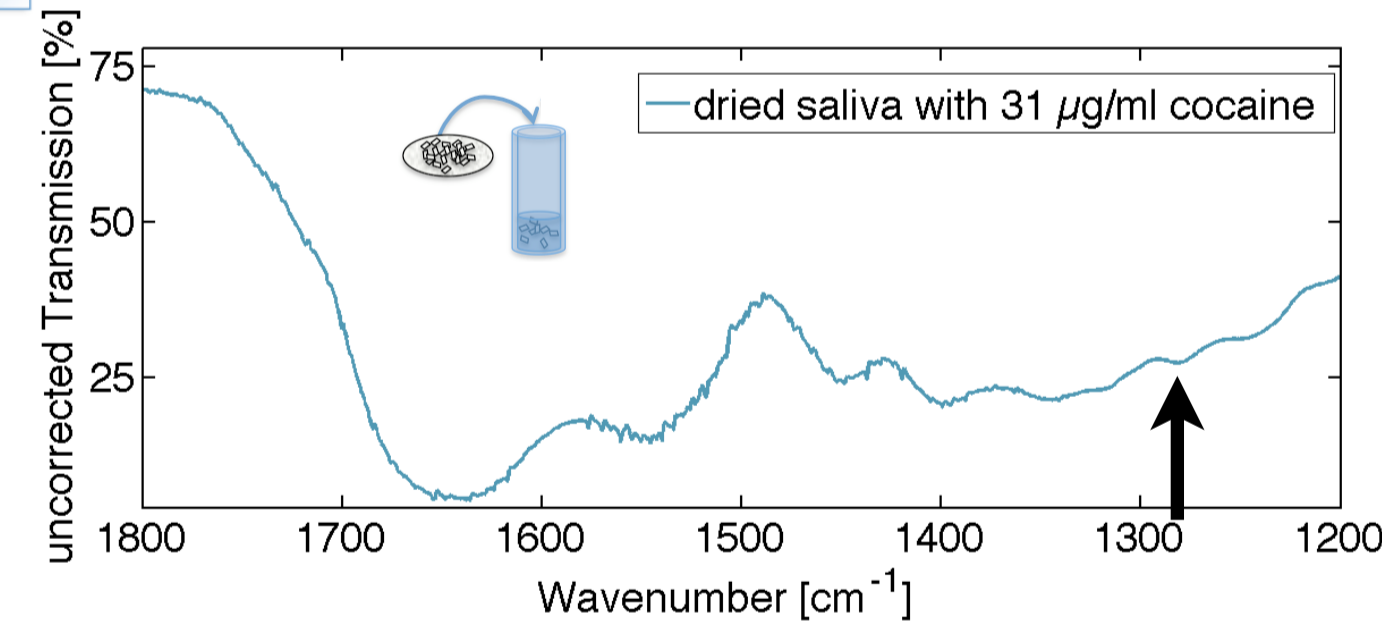


- Black: suspension of water and cocaine flakes (@1260 µg/ml) after water background subtraction
- Yellow boxes indicate the areas of strong cocaine absorption

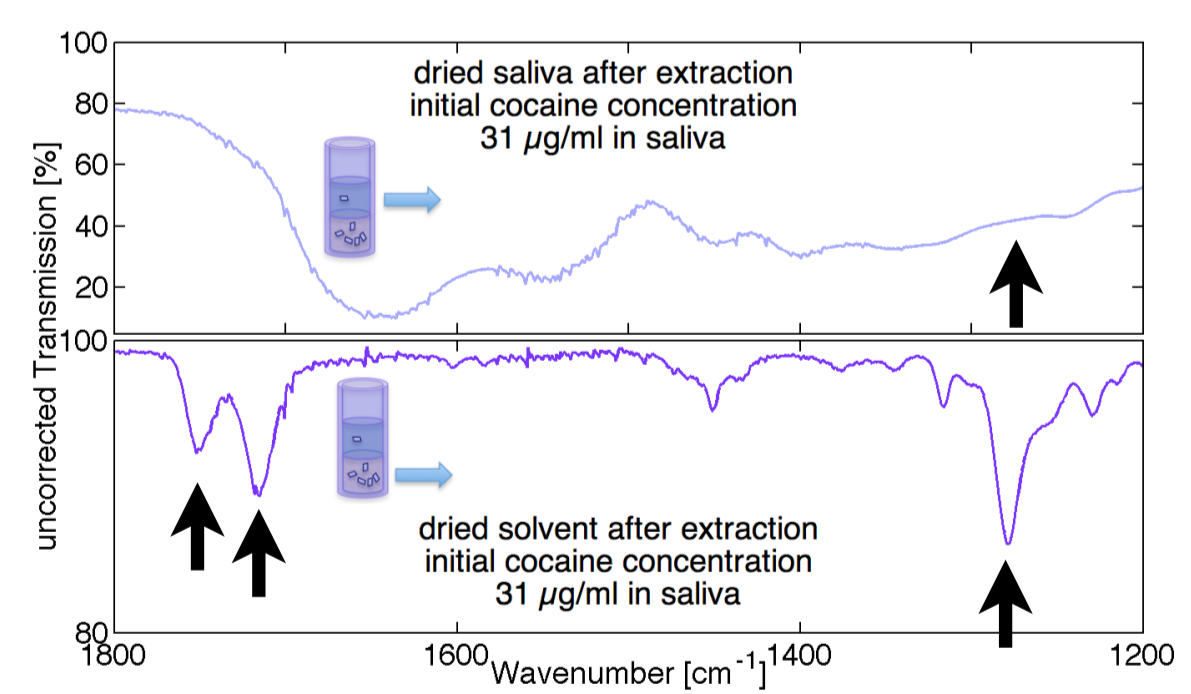
Sample extraction and spectra



Spectrum of dried saliva: **without cocaine and spiked with cocaine**; the appearance of the **cocaine peak** around 1280 cm⁻¹ is clearly visible and marked with a black arrow (peaks at ~1740 cm⁻¹ only visible in the ratio)

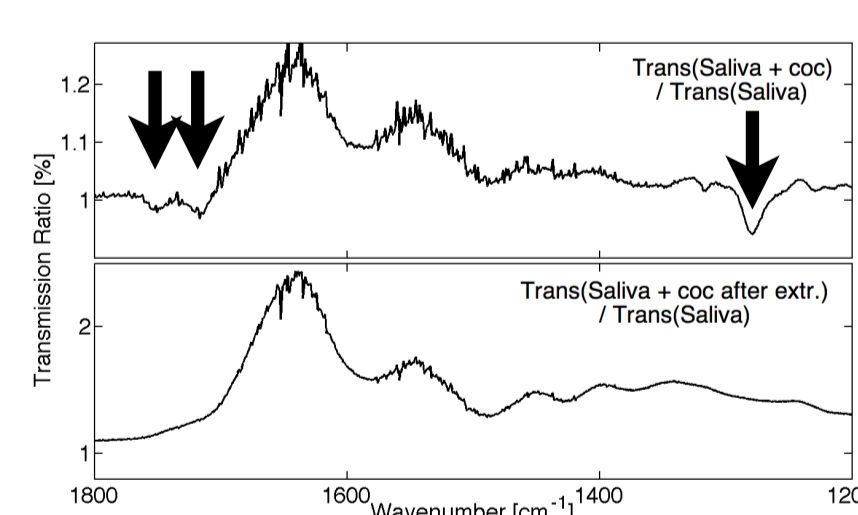


FTIR-spectra of **saliva** and **solvent** phase after the extraction: => cocaine peaks in saliva disappeared, but appear in **solvent**
best tested solvent for extraction: Tetrachloroethylene

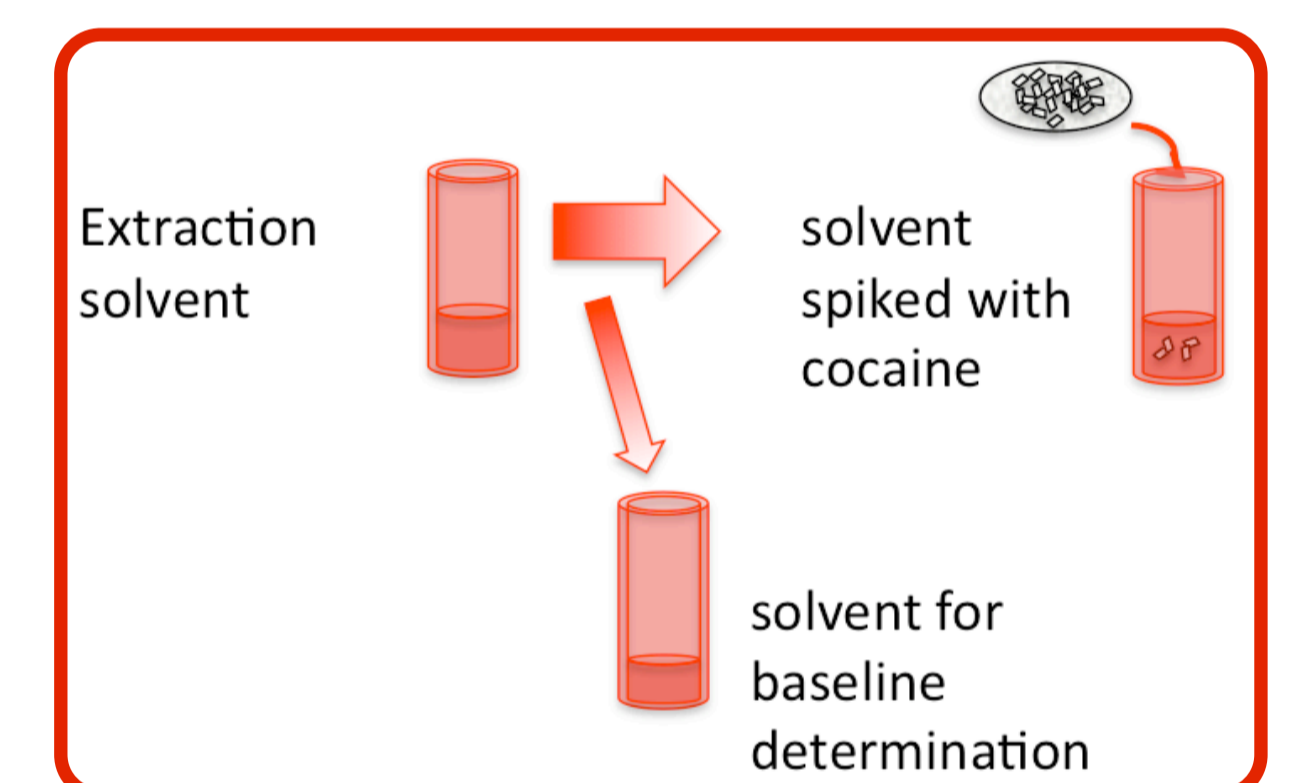


Ratio of several transmission spectra:

- values <1: cocaine absorption
- values >1: difference in drying patterns

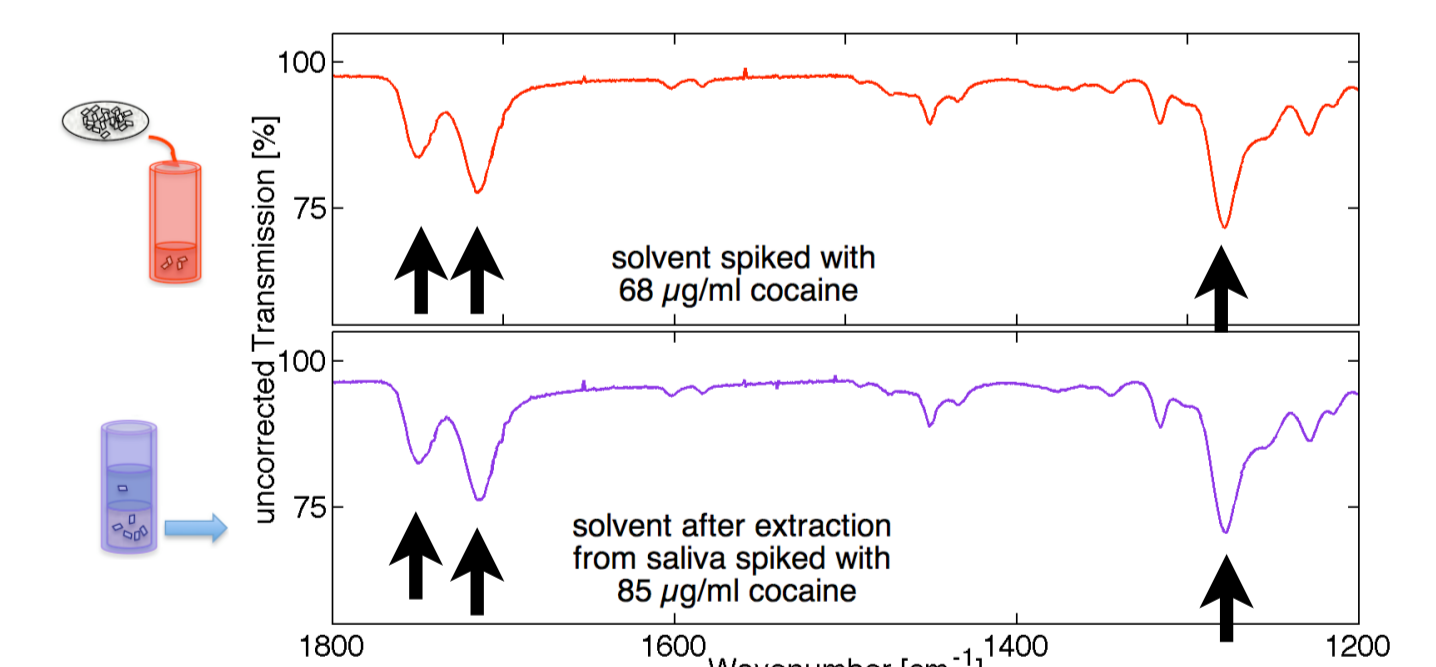


Reference samples and spectra

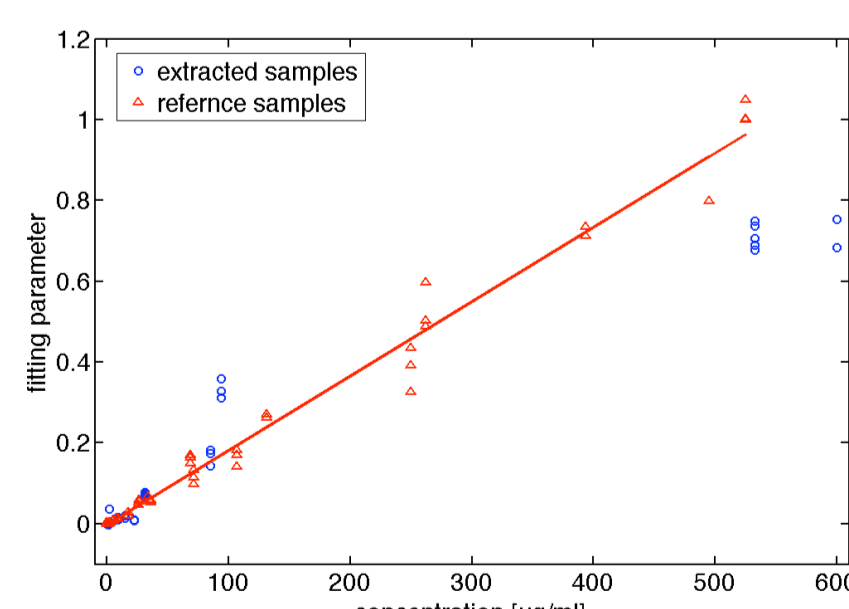


For comparison and determination of extraction efficiency => investigation of **reference samples**

- spectrum of dried **reference sample** and **spectrum after extraction almost identical**
- **extraction efficiency >80%**

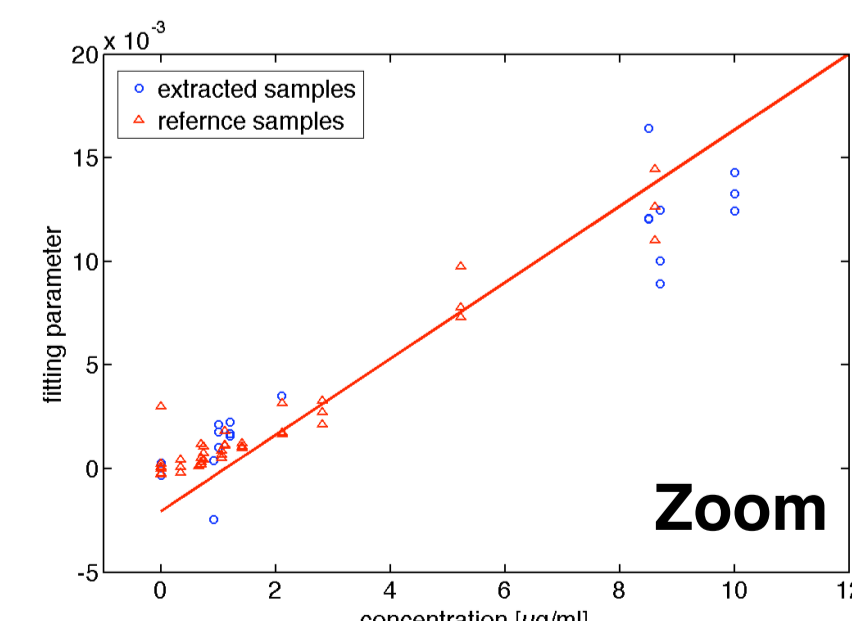


Extraction efficiency determined with the FTIR

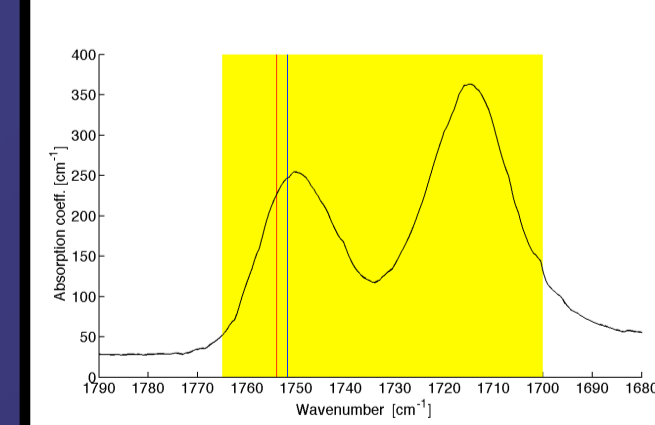


A low noise cocaine spectrum with subtracted baseline was used for the fit. Fit parameters vs. concentration => linear relation (r=0.993)

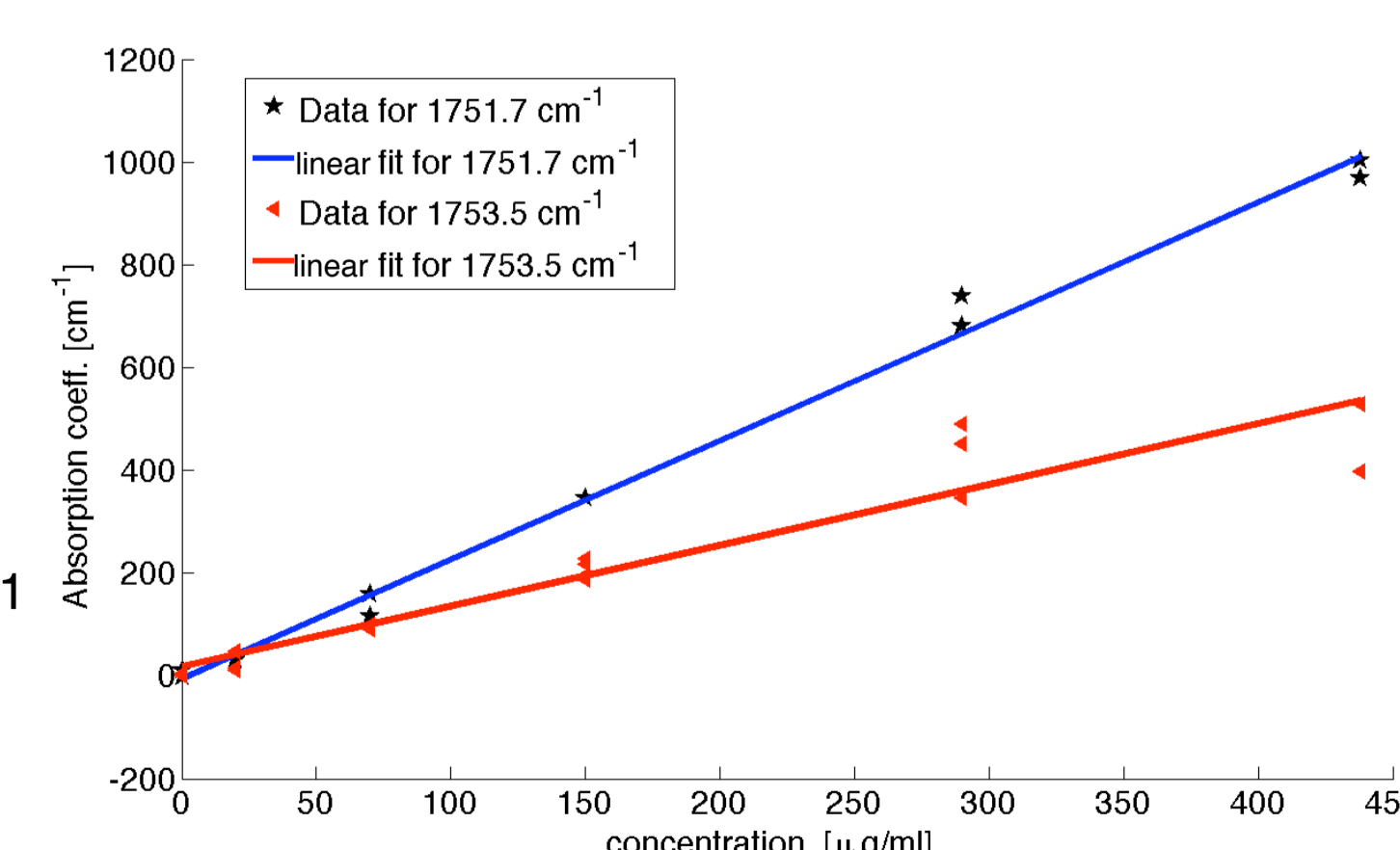
Extraction efficiency >80% (often almost 100% within error) (current LOD ~2 µg/ml)



QCL measurement



Chosen wavelengths 1751.7 and 1753.5 cm⁻¹ with respect to the cocaine spectrum



Measurements of reference samples with one wavelength

Laser instabilities yield a current **limit of detection** with the **QCL** of ~20 µg/ml

Conclusions and outlook

- Successful **one-step extraction** of Cocaine, Cocaine.HCl and several metabolites from saliva
- **Improved limit of detection** with the help of **extraction** and **dried** samples (semi-quantitative results): currently ~2 µg/ml

- Measurements with QCL at 1751.7 and 1753.5 cm⁻¹ (supplied by Yargo Bonetti, ETH) very promising
- Combination of tunable QCL waveguide and microfluidics in progress

Literature

[1] Walsh et al., J. Anal. Toxicol 27, 429 (2003)
[2] Hans et al., Drug Testing and Analysis, DOI: 10.1002/dta.346

[3] Jenkins et al., J. Anal. Toxicol. 19, 359 (1995)
[4] Jufer et al., J. Anal. Toxicol. 30, 458 (2006)
[5] Moolchan et al., J. Anal. Toxicol. 24, 458 (2000)
[6] E.J. Cone et al., Clinical Chemistry 40(7), 1299 (1994)

Media presence:

15th Feb 2012 Nr. 38; 9th Jan 2012; News 9th Jan 2012, Radio Top; ETH Life 6th Jan 2012; swissinfo.ch 9th Feb 2012;...