

Analysis of 3-hydroxybenzo[a]pyrene in urine as a biomarker of exposure for benzo[a]pyrene in smokers and users of potentially reduced risk products

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3-Hydroxybenzo[a]pyrene – Biomarker of Exposure to Benzo[a]pyrene in Urine

- Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH), formed during the incomplete combustion of organic materials
- BaP is classified as a human carcinogen (IARC Class 1)
- BaP is included in the established list of harmful and potentially harmful constituents in tobacco products and smoke
- Urinary 3-Hydroxybenzo[a]pyrene (3-OH-BaP) is frequently determined as a biomarker of BaP exposure
- High 3-OH-BaP concentrations were found in occupationally exposed populations (higher than 200 ng/g creatinine)¹⁻³
- **Development of a highly sensitive method for human biomonitoring in non-occupationally exposed populations based on a previously published method⁴**

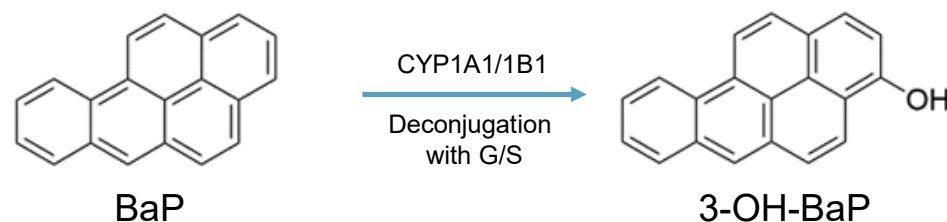


Figure 1: Metabolism of BaP

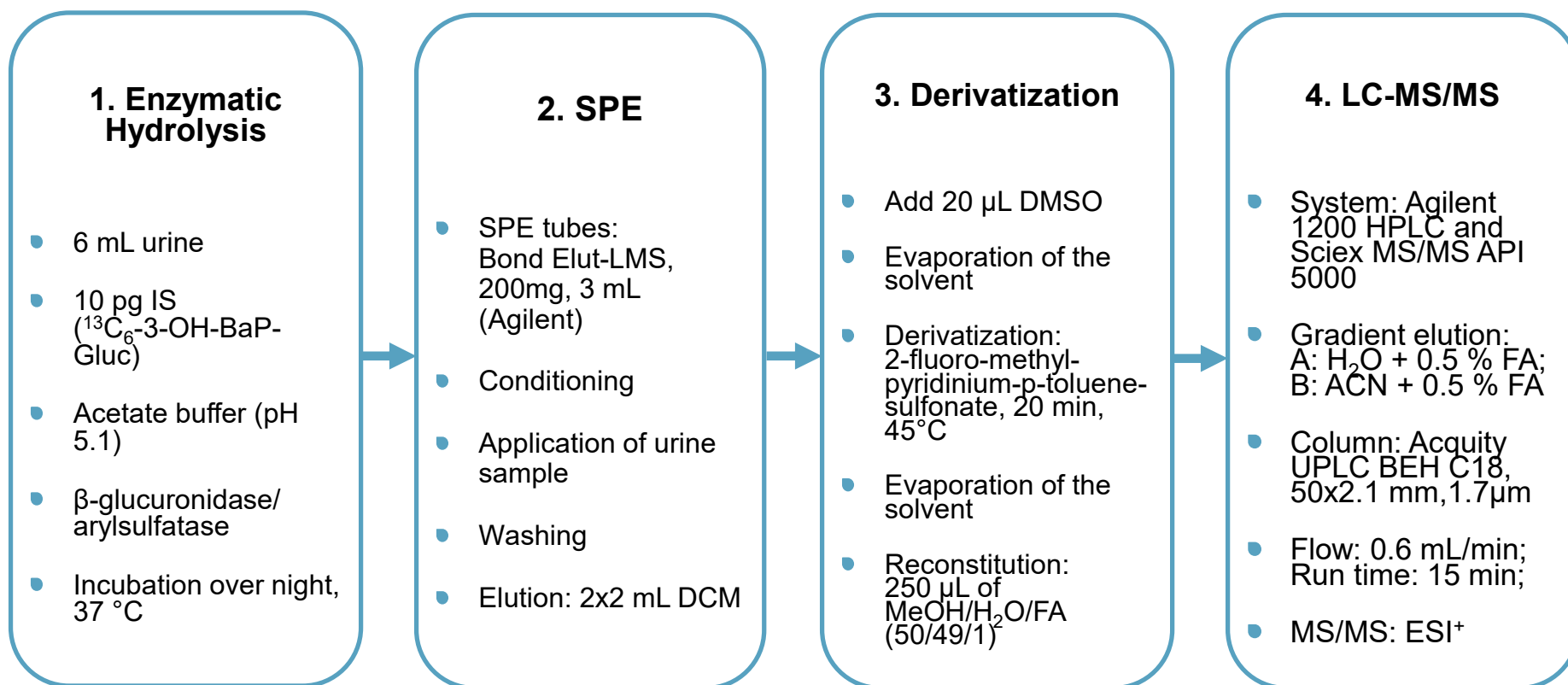
¹ Förster, K., et al. 3-Hydroxybenzo[a]pyrene in the urine of workers with occupational exposure to polycyclic aromatic hydrocarbons in different industries. *Occup. Environ. Med.* 2008, **65**, 224-229.

² Gündel, J., et al. High-performance liquid chromatographic method with fluorescence detection for the determination of 3-hydroxybenzo[a]pyrene and 3-hydroxybenz[a]anthracene in the urine of polycyclic aromatic hydrocarbon-exposed workers. *J. Chromatogr. B. Biomed. Sci. Appl.* 2000, **738**, 47-55.

³ Barbeau, D. et al., Relevance of Urinary 3-Hydroxybenzo(a)pyrene and 1-Hydroxypyrene to Assess Exposure to Carcinogenic Polycyclic Aromatic Hydrocarbon Mixtures in Metallurgy Workers. *Ann. Occup. Hyg.* 2014, **58**, 579-590.

⁴ Sarkar, M., et al. Evaluation of biomarkers of exposure in adult cigarette smokers using Marlboro Snus. *Nicotine Tob. Res.* 2010, **12**, 105-116.

Quantification of 3-OH-BaP in Urine – Preparation and Analysis



Quantification of 3-OH-BaP in Urine – Method Validation

- Validation according to FDA guidelines
- LOD: 16.7 pg/L
- LLOQ: 50 pg/L (accuracy: 104 %; precision: 8.4 % CV)
- Calibration range: 50–3321 pg/L

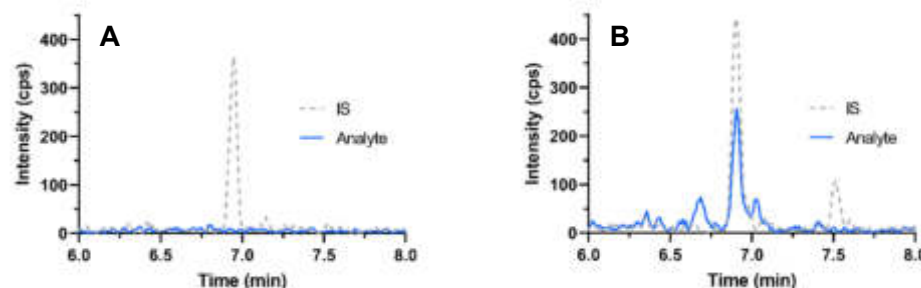


Figure 2: MRM chromatograms of 3-OH-BaP (m/z 360.2 $\rightarrow$ 251.1) and the IS $^{13}\text{C}_6$ -3-OH-BaP (m/z 366.2 $\rightarrow$ 257.1) of A: non-smoker urine and B: smoker urine

Table 1: Method validation results for precision, accuracy, and matrix effect

	Low (200 pg/L)	Medium (640 pg/L)	High (1600 pg/L)
Precision, intra-day	10 % CV	4.3 % CV	6.7 % CV
Precision, inter-day	10 % CV	14 % CV	14 % CV
Accuracy	102 %	92.6 %	110 %
Matrix effect	+31 %; +25 % (IS)	-	+43 %; +48 % (IS)

Clinical Study – Trial Design

- Aim: Exposure assessment to BaP in smokers and users of potentially reduced risk products in non-occupationally exposed, healthy subjects compared to non-users
- Confinement over three days and diet-controlled clinical study
- Collection of all urine voids on each study day and preparation of 12h-urines for analysis

Table 2: Main characteristics of the study population

User group	N (m/f)	Age (years) Mean $\pm$ SD	BMI Mean $\pm$ SD	24h-urine ¹ (mL) Mean $\pm$ SD
Cigarette (CC)	10 (6/4)	35.1 $\pm$ 9.1	26.0 $\pm$ 3.9	2891 $\pm$ 828
Heated tobacco products (HTP)	10 (6/4)	36.1 $\pm$ 12	25.5 $\pm$ 3.2	2685 $\pm$ 1300
Oral tobacco (OT)	10 (9/1)	28.1 $\pm$ 8.2	25.9 $\pm$ 4.2	2638 $\pm$ 1290
E-cigarette (EC)	10 (6/4)	38.4 $\pm$ 14	23.5 $\pm$ 2.7	1627 $\pm$ 664
Nicotine replacement therapy (NRT)	10 (5/5)	35.3 $\pm$ 15	25.5 $\pm$ 3.5	1602 $\pm$ 802
Non-users (NU, control)	10 (6/4)	32.9 $\pm$ 8.8	24.7 $\pm$ 3.2	2475 $\pm$ 936

¹ Urine voids collected on the last study day (6 pm until 6 pm)

Clinical Study – Results

- Analysis of 12h-urines of day 3 (longest time of confinement/control in the study)¹
- CC: 60 % of study samples with concentrations >LLOQ
- HTP, OT, EC, NRT, and NU: 99 % of the study samples showed concentrations <LLOQ
- Mean value of CC smokers²: 149 pg/24 h
- Mean value of the other groups²: 40.7–67.1 pg/24 h
- Significantly higher concentrations in CC smokers
- **Application of the method allowed differentiation between smokers of CCs and users of other nicotine products as well as NUs**

¹ 12 h periods: from 6 pm to 7 am and from 7 am to 6 pm

² All values below the LLOQ were set to LLOQ/2 (25 pg/L).

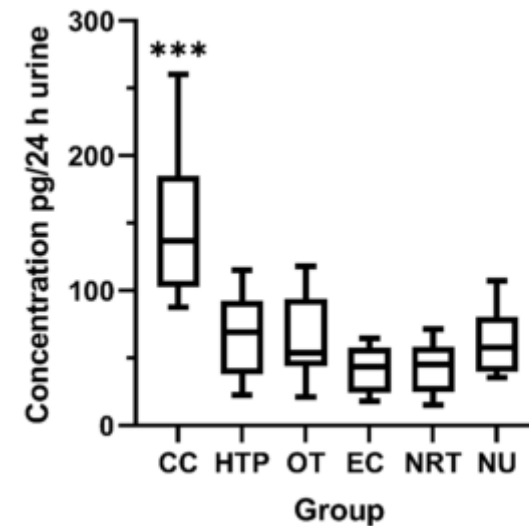


Figure 3: Box plots for urinary 3-OH-BaP excretion (pg/24h). Significant differences between the cigarette smokers (CC), the other user groups (HTP, OT, EC, and NRT), and the non-users (NU) were evaluated using the non-parametric Mann-Whitney U test (***: p value < 0.002)

Clinical Study – Results

- Moderate correlation (Pearson) of 3-OH-BaP excretion with cigarettes per day and good correlation with cotinine excretion in smokers
- Correlation between 3-OH-BaP and cotinine statistically significant ($p < 0.05$)

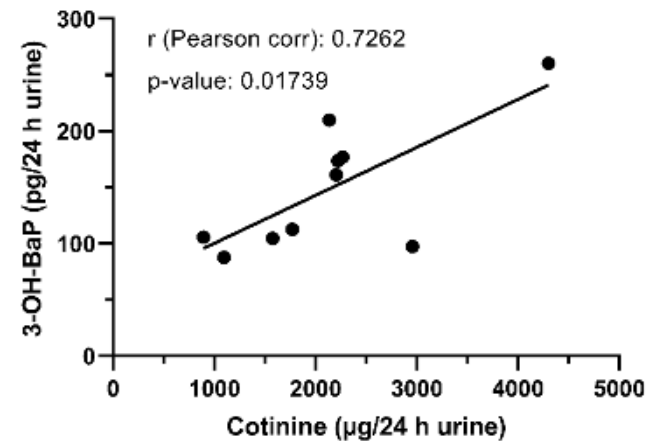
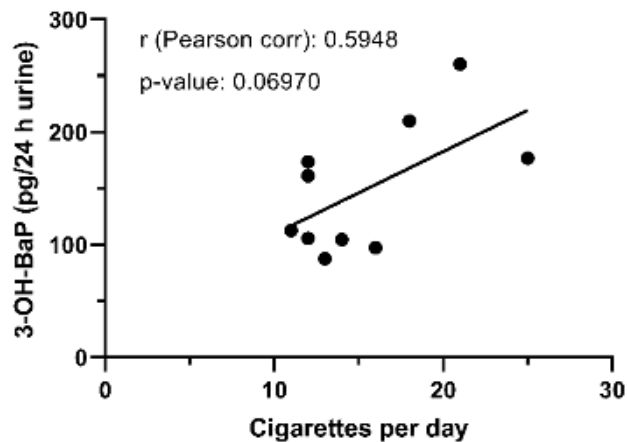


Figure 4: Linear regression for 3-OH-BaP and cigarette consumption per day (left), and for 3-OH-BaP and cotinine (right) of smokers on Day 3 (10 subjects).

Conclusion

- Developement of a highly sensitive and high-throughput LC-MS/MS method for the quantification of 3-OH-BaP in urine suitable for the determination in non-occupationally exposed populations
- Method validation according to FDA guidelines revealed excellent accuracy/precision with a very low LLOQ (50 pg/L)
- Application of the method to 12h-urine samples collected in a controlled clinical study with smokers of CCs, users of ECs, HTPs, OT, NRT, and NUs
- 3-OH-BaP mostly not quantifiable in users of potentially reduced risk products (similar to NU) under the confined conditions
- Smokers of CCs showed significantly higher 3-OH-BaP excretion as compared to all other user groups and the NUs and moderate to good correlation to cigarette consumption and urinary cotinine levels
- 3-OH-BaP is a suitable biomarker to assess the exposure to the carcinogen BaP by cigarette smoke exposure

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