A sensitive GC–MS/MS method for the quantification of benzo[a]pyrene tetrol in urine

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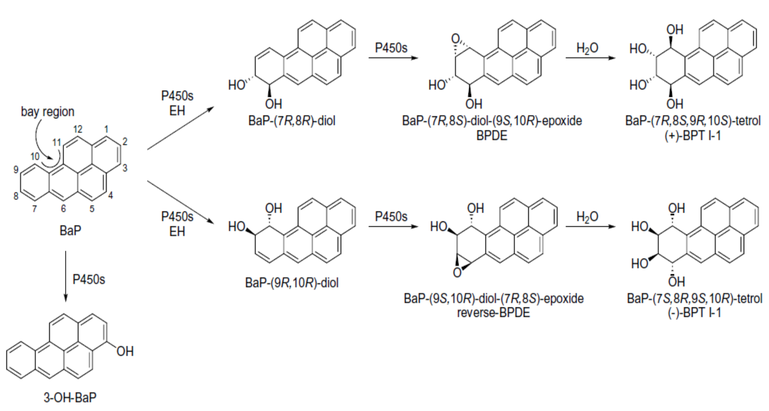
Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants formed during the incomplete combustion of organic matter such as tobacco. Among these, benzo[a]pyrene (BaP) has been classified as a known human carcinogen. It unfolds its effect through metabolic activation to BaP-(7R,8S)-diol-(9S,10R)-epoxide (BPDE), the ultimate carcinogen of BaP. Here we present a simple and highly sensitive GC–NICI–MS/MS method for the quantification of urinary BaP-(7R,8S,9R,10S)-tetrol ((+)-BPT I-1), the hydrolysis product of BPDE. The method was validated and showed excellent results in terms of accuracy, precision, and sensitivity (lower limit of quantification (LLOQ): 50 pg/L). In urine samples derived from users of tobacco/nicotine products and non-users, only consumption of combustible cigarettes was associated with a significant increase in BPT I-1 concentrations. Levels of users of potentially reduced-risk products as well as non-users were all below the LLOQ. In addition, the urine levels of six occupationally exposed workers were analyzed and showed the highest overall concentrations of BPT I-1 (844.2 ± 336.7 pg/L). Moreover, correlation with 3-hydroxybenzo[a]pyrene (3-OH-BaP), the major detoxification product of BaP, revealed higher levels of 3-OH-BaP than BPT I-1 in almost all study subjects. Despite the lower levels, BPT I-1 can provide more relevant information on an individual's cancers risk. In conclusion, BPT I-1 is a suitable biomarker to distinguish not only cigarette smokers from non-smokers but also from users of potentially reduced-risk products.



Detoxification pathway of BaP to 3-OH-BaP and metabolic activation of BaP to the ultimate carcinogen BPDE and its subsequent convertion to the enantiomeric tetrols (+)-BPT I-1 and (–)-BPT I-1. EH, epoxide hydrolase; P450, cytochrome P450

Ref: F. Pilz, A. Gartner, N. Pluym, G. Scherer and M. Scherer: Anal Bioanal Chem 2024; DOI: 10.1007/s00216-024-05233-9

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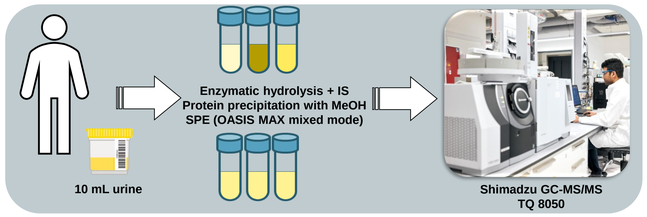
GC-NICI-MS/MS analysis of B[a]P-tetrol



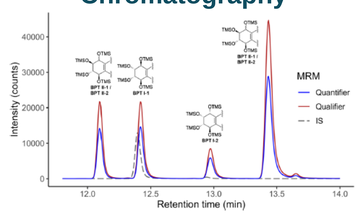
Sample work-up

- 10 mL Urine + 5 mL acetate buffer + 20µL (+)-BPT I-1-d_8 (IS) Enzymatic hydrolysis over night
- 4 mL MeOH → Centrifugation
- SPE on a OASIS MAX cartrides.
- Evaporation to dryness, reconstitution in 200µL MeOH

GC-NICI-MS/MS

- Rxi™-5 ms column (Restek)
- 1.26 ml/min He as carrier gas
- Split/splitless injection (1µL)
- T gradient elution
- Negative chemical ionization MRM mode

Chromatography



- MRM chromatograms obtained from the analysis of tetra-TMS BPT ethers (quantifier, m/z 446.2 → 255.1 and qualifier, m/z 446.2 → 284.1) and the IS (±)-BPT I-1-d8 (m/z 454.2 → 263.1)
- (±)-BPT I-1 (12.42 min), (±)-BPT I-2 (12.97 min), (±)-syn-BPDE, (±)-BPT II-1 and (±)-BPT II-2 (12.10 min and 13.43 min).

Method Validation Results

Analyte	Precision / %CV	Accuracy / %	LLOQ / fg/mL	Range / fg/mL	Recovery / %
BPT I-1	3.5-12.3	91-117	50	50-2500	BPT I-1

- Full method validation according to ICH M10 proved accuracy, precision and sensitivity of the method
- Method suitable for clinical study sample analysis

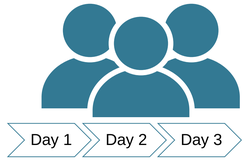
Method accredited DIN EN ISO/IEC 17025:2018 DAkkS Deutsche Akkreditierungsstelle D-PL-18356-01-00

GLP GOOD LABORATORY PRACTICE CERTIFIED

Method application

Controlled Clinical Study

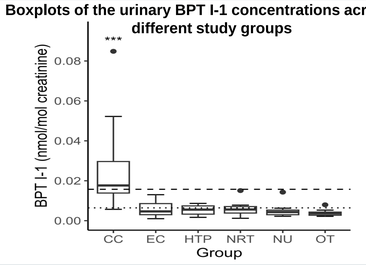
- 10 subjects per group
- 3 days of confinement
- 5 nicotine product user groups



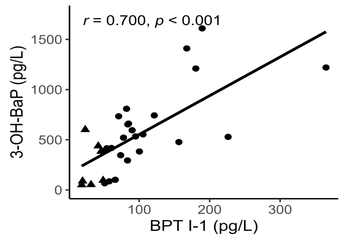
Ref: F. Sibul et al: Anal Bioanal Chem 2024; Contemp Clin Trials Commun. 2021;22: 100794

Results

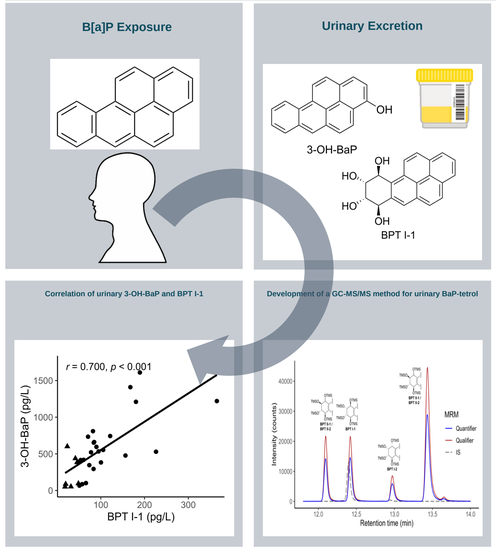
Boxplots of the urinary BPT I-1 concentrations across six different study groups



Correlation analysis BPT I-1 vs. 3-OH-B[a]P in N=31 Smokers



Summary & Conclusion



- **A new method for urinary BPT I-1 was successfully developed and validated according to ICH M10 guideline**
- **The method is highly sensitive, precise, robust and covers a broad concentration range, allowing its applicability in future clinical studies**
- **The methods was applied to urine samples derived from a clinical study with 5 different nicotine product user groups and non-user**
- **Smokers (CCcoul clearly distincted from all other groups**
- **Correlation of BPT I-1 with 3-OH-B[a]P was moderate to strong for all urine samples of smoking subjects in the study (r=0.7)**
- **Since BPT I-1 is the hydrolysis product of BPDE, the ultimate carcinogen of BaP, the biomarker is more suited to evaluate the PAH-related health risk than other metabolites such as 3-OH-BaP.**