



## Analysis of DNA adducts: Development of a high-performance analytical platform to meet challenges in human health

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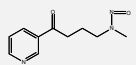
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
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### Introduction

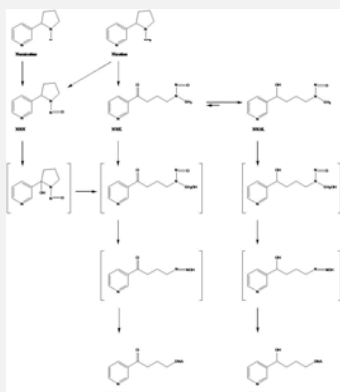
Tobacco-specific nitrosamines (TSNA)

- 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)  and

- N'-nitrosornicotine (NNN) 

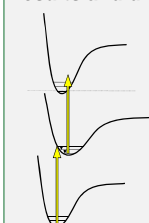
have shown by far the highest specificity for exposure to tobacco and tobacco smoke.

- Based on experimental data in animals and human tissues and results from biomonitoring, both NNK and NNN have been classified as human carcinogens (group 1) by the International Agency for Research on Cancer.
- However, to exert their carcinogenic effect, NNK and NNN require metabolic activation to highly reactive intermediates giving rise to protein and DNA adducts.
- A biomarker of the biologically effective dose of the tobacco-specific nitrosamines would be the amount of a reactive metabolite bound to macromolecules either in target or surrogate tissue. One of the metabolic activation pathways from both NNK and NNN lead to highly reactive species giving rise to hemoglobin and DNA adducts:



- Contrary to the expectation, the POB adducts of hemoglobin or DNA do not show the expected specificity for exposure to tobacco and tobacco smoke.
- According to results for urinary NNAL and its glucuronides, differences between smokers and passive smokers should be more than 50-fold.
- However, POB-hemoglobin adducts differed less than threefold. Even the highest difference of about sevenfold, seen in lung DNA adducts of smoking and nonsmoking lung cancer patients, was far below the expected values.
- There is a hypothesis of Richter et al. that dietary myosmine should be an important additional source of POB-DNA adducts.
- Richter et al. have demonstrated a sensitive method for POB-adducts based on derivatization with pentafluorobenzoyl chloride and high-resolution mass spectrometer with a detection limit of 4.6 fmol.

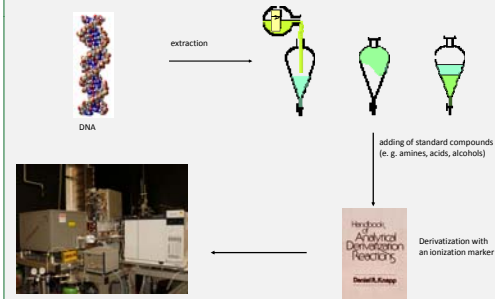
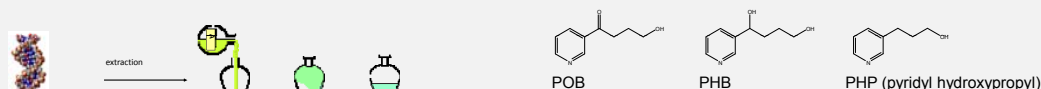
### Results and discussion



Atmospheric-pressure laser ionization (APLI), recently developed by the groups of Analytical and Physical Chemistry at the University of Wuppertal, shows an outstanding sensitivity for moderately to non-polar aromatic compounds. This selectivity towards aromatic compounds arises from the ionization mechanism of APLI: Multiphoton excitation of matrix compounds is minimized by adjusting the laser power density close to the threshold of resonantly enhanced (1+1) multiphoton excitation. Linear absorption of most matrix compounds becomes negligible when photons with a wavelength of 248 nm are used for excitation. Readily available small-footprint excimer lasers are sufficiently powerful light sources. With respect to efficient resonant two-photon ionization, the spectroscopic features of aromatic hydrocarbons are rather unique: They display strong linear absorption cross sections at 248 nm, long-lived intermediate electronic states, and highly vertical ionization transitions. Since hardly any other compound class exhibits such features, APLI is specific for arenes.

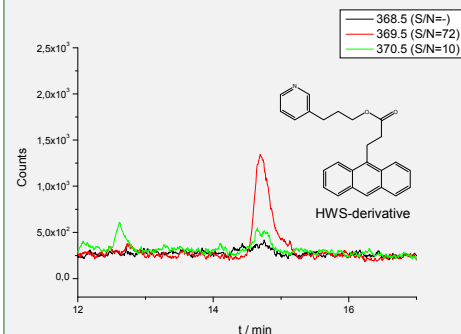
In this work we present a derivatization strategy that facilitates selective ionization of DNA adducts by GC-APLI-(TOF)MS with a good sensitivity. With this procedure the analytes are detected by the mass analyzer without noticeable interference from the matrix.

To reduce the cost of the optimization a substitute (PHP) for the expensive POB and PHB was used:



### Derivatization procedure

- 500  $\mu$ L CT-DNA-solution (2 mg/mL) was added with 5  $\mu$ L 10  $\mu$ M PHP
- mixture was added with 105  $\mu$ L 4 M HCl and hydrolyzed 3 h at 80  $^{\circ}$ C
- the hydrolysate was added with 640  $\mu$ L 1 M NaOH and 100  $\mu$ L phosphate buffer (0.5 M, pH 7.2)
- the solution was extracted with dichloromethane 3 x 300  $\mu$ L  $\text{CH}_2\text{Cl}_2$
- dichloromethane (DCM) was evaporated to dryness and the sample was derivatized with 3  $\mu$ L dimethyl aminopyridine (100 mM), 20  $\mu$ L HWS (8 mg/mL ionisation marker), 50  $\mu$ L DCM and 50  $\mu$ L DCC (100 mM, carbodiimide) over night at 35  $^{\circ}$ C



The chromatogram shows the analysis of 3,3 fmol of derivatized PHP on GC-column. In future we will

- Increase in sensitivity by further optimization of the derivatization procedure (a factor of 10 to 100 should be possible)
- Analysis of real samples with the finally developed analytical method
- Derivatization of 5-hydroxymethylcytosine which is discussed as a further epigenetic marker with the same method

### Literature

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