DRIED BLOOD SPOT-BASED ANALYSIS OF THE CYANOETHYLVALINE-ADDUCT OF HEMOGLOBIN FOR SMOKING ASSESSMENT

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Cigarette smoke contains more than 4000 chemicals, one of which is acrylonitrile. Acrylonitrile is a chemical substance widely used in industry for different purposes like fabrication of synthetic fibres, household articles and resins. Its toxicity is of major concern not only during chronic exposure but particularly also during acute poisoning. Smokers are exposed chronically, while acute poisoning can happen in disasters with spill-over of acrylonitrile, as was the case in the train disaster in Wetteren in 2013.

Here, we present a new dried blood spot (DBS)-based method to screen for exposure to acrylonitrile. When acrylonitrile enters the bloodstream, it binds covalently to hemoglobin, thus forming an adduct, the N-terminal cyanoethylvaline-adduct (CEV). In contrast to nicotine and cotinine, which only have a short half-life, this CEV adduct remains present for months, offering a wide window of detection. The use of DBS offers many benefits since it is a minimally invasive sampling approach for the patient, which is especially relevant for newborns.

Existing methods to screen for the CEV adduct are based on a modified Edman degradation. This procedure allows specific detachment and isolation of N-substituted N-terminal valine as a thiohydantoin derivative. However, these methods are lengthy (about 16 hours of derivatization) and require several milliliters of blood.

In our method, we implemented microwave-based on-spot derivatization of DBS and further optimized the degradation. In the optimized procedure fluorescein isothiocyanate(FITC) is added onto a 6 mm DBS punch and derivatization is performed for 15 minutes in the microwave at 300 W. Further sample clean-up is done by solid phase extraction. For the measurement of this adduct an LC-MS/MS/MS method was developed. In this MS/MS/MS method the motherion is FITC-CEV with m/z 542 which fragments to a daughterion of m/z 499, which in turn fragments to a new daughterion of m/z 374. This method was already successfully applied on genuine smoker samples.

Validation will be performed based on US FDA and EMA guidelines for bioanalytical method validation. This validation will comprise selectivity, carry-over, LLOQ, linearity, precision, accuracy, matrix effect, recovery, stability and incurred sample reanalysis. Carry-over will be analyzed by running blanks after samples with high concentrations of the analyte. The linearity will be examined by using a calibration curve made out of blank blood spiked with a custom-made CEV-peptide at different concentrations. Precision will be determined by analyzing the same sample several times. The matrix effect and recovery will be tested by analyzing blood of different hematocrits, while the stability will be tested at different temperatures (room temperature, 4 °C and -20 °C) for several months.

Following validation, this methodology will be applied on DBS from smokers, passive smokers and non-smokers, to evaluate the potential of this novel approach to assess (historical) smoking behavior.