Poster

[P27-7] P27-7: Assay

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[P27-7-7] Measurement of nicotine, cotinine and OH-cotinine in exhaled breath with LC-MS/MS

Shahid Ullah[1], Olof Beck[2] (1.Karolinska University Hospital, 2.Karolinska University Hospital)

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Background

Nicotine is a natural stimulant and an alkaloid dominantly found in different plants, especially tobacco plant. It is considered to be highly addictive, and getting overdose to the mammals may lead to lethal consequences. The most common matrices for analyzing nicotine and its metabolites in human are blood, plasma, urine and saliva for the diagnosis of poisoning and medical investigation. Also, particles in exhaled breath contain non-volatile substances and could be used an alternative matrix to them. In this study, an LC-MS/MS method was developed to analyze nicotine, cotinine and OH-cotinine in human exhaled breath particles.

Methods

Particles in Exhaled breath were collected in a commercial sampling device called Sensabues (Sensabues AB, Sollentuna, Sweden). During sampling of exhaled breath containing microparticles pass through a mouth-piece and subsequently collected to a polymer filter with a diameter of 30 mm inside the device. After extraction with 6 ml MeOH samples were analyzed by UHPLC-MS/MS. Mass spectrometer was operated in ESI positive mode. The chromatographic separation was achieved on an acquity UPLC BEH phenyl column (2.1 ×100 mm, 1.7 m) with injection volume of 2 L at a mobile phase flow rate 450 l/min in a gradient mode. Column oven temperature was 60 ˚C. Mobile phase A consisting of water and a mobile phase B consisting of MeOH with 4 mM ammonium formate and 0.05% ammonia in both A and B.

Results

The method detection limits were 3 pg/filter for all three substances. Excellent linearity (r^2 0.99) was achieved with six point calibration levels with real sample spiked over the concentration range 10 –20000 pg/filter for all analytes. Method recovery with two concentrations at 10 pg/filter and 20000 pg/filter were 100 –130% and 100 –105%, respectively, for all three substances. During method application, a preliminary screening experiment suggests that nicotine and its metabolites could be detected in exhaled breath 6 hrs (or longer) after the smoking or using snuff with the concentration range 30 –190 pg/filter.

Conclusions

Exhaled breath is a valuable matrix for determination of nicotine and metabolites after smoking. The LC-MS/MS method developed here is very sensitive and provides easy sample collection procedure. This method could be used for the purpose of monitoring nicotine abstinence, pre-employment and health insurance screening program.