Rapid and selective analysis of nicotine and cotinine in urine by using HPLC-UV with post-column photo-irradiation

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INTRODUCTION AND AIM: In recent times, much attention is being paid by forensic toxicologists to the determination of objective markers of substance abuse, since litigation concerning child custody, compensation from health insurances, physical fitness to specific jobs etc based on hypotheses of drug abuse has considerably increased. Nicotine is rapidly and extensively metabolized in humans and the first step of its biotransformation is oxidation to cotinine [1]. Both substances can be used as objective markers to determine exposure to tobacco smoke [2]. Notwithstanding the availability of established GC-MS and HPLC-MS methods for nicotine and cotinine analysis, there is still a need for rapid, robust and low cost methods for routine use, which however must meet the highest standards of selectivity.
The present work was aimed at the development of a simple and selective high-performance liquid chromatography (HPLC) method to determine nicotine and cotinine in human biosamples.

MATERIALS AND METHODS: Liquid-liquid extraction of 5 ml of urine samples was performed by using ToxiTubes A (Varian, Lake Forest, CA). The organic phase was dried under air stream and reconstituted in 500 µL of water. The analysis was carried out by HPLC (Shimadzu, Duisburg, Germany) in isocratic reverse-phase separation mode, using a polystyrene/divinylbenzene column (PLRP-S, Polymer Labs, Church Stretton, UK). The mobile phase was composed of 20 mM ammonium acetate at pH 11 added with tetrahydrofuran and acetonitrile (83/2/15; v/v/v); the flow rate was 0.5 ml/min. UV detection at 390 nm wavelength was performed after post-column photochemical irradiation in a photochemical reactor (BeamBoost, ICT, ...) employing a germicidal 8 W UVC mercury lamp and a 10 m knitted Teflon® tubing (I.D. = 0.30 mm).

RESULTS AND DISCUSSION: With the proposed method, UV determination of nicotine and cotinine could be accomplished by UV absorption at 390 nm wavelength, instead of 260 nm, as is typically used in other direct methods based on HPLC-UV. The photoirradiation-induced shift to higher wavelengths of nicotine and cotinine UV absorption spectra, conceivably caused by dimerization, provides a high increase of detection selectivity. Flat baselines without any interferences were observed in the analysis of blank urines. Extraction recoveries were 84-88% for nicotine and 78-74% for cotinine. The limit of detection (LOD) in biological fluids, calculated with at a signal to noise (S/N) ratio of 3, was 0.02µ g/ml for nicotine and 0.05µ g/ml for cotinine. In urines from real smokers, the concentrations of nicotine and cotinine were 0.02-2.00 µg/ml and 0.03-0.40. µg/ml, respectively. In urines from non-smokers only minor traces of nicotine could be detected with the present method.

REFERENCES:

1 F. Ceppa et al., J. Chrom B, 2000, 115-122.

2 NL. Benowitz et al., Nicotine & Tobacco Research, 2002, 149-159.

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