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Solid-phase extraction of nicotine and its metabolites for high-performance liquid chromatographic determination in urine

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Abstract

A solid-phase extraction, using Extrelut-1 glass columns, has been applied to urine samples of both passive and active smokers for high-performance liquid chromatographic determination of nicotine and its metabolites cotinine and *trans*-3′-hydroxycotinine. Chromatography was performed using a reversed-phase LC_8DB column and a mobile phase consisting of water–acetonitrile (80:9, v/v) containing 5 ml triethylamine, 670 mg/l sodium heptanesulphonate, and 0.034 *M* each of K_2HPO_4 and citric acid (pH 4.4), at a flow-rate of 1.6 ml/min. The results obtained indicate that solid-phase extraction is a reliable and quick procedure which can be applied also to other nicotine metabolites.

Recently, we described high-performance liquid chromatographic (HPLC) methods with ultraviolet detection for the determination of nicotine and its metabolites cotinine and *trans*-3′-hydroxycotinine [1,2]. The assay was performed on serum samples, since serum steady-state levels of nicotine metabolites were used to estimate the daily intake of nicotine after cigarette smoking.

Here we report a slight modification in the solid-phase extraction procedure which allows application to urine samples of both passive and active smokers. The feature of this application is that for the first time solid-phase extraction is performed on urine samples.

Extrelut-1 glass columns (Merck, Bracco, Milan, Italy), used for the extraction procedure, are pre-packed columns filled with 700 mg diatomaceous earth. They have to be preconditioned to improve sample clean-up; if the preconditioning is done the day before the extraction, the column is nearly dry on the day of extraction and sample loss, which occurs with wet columns, is avoided.

A 200-μl aliquot of urine, with 200 μl of N-ethylnorcotinine (10 μg/ml aqueous solution) added as an internal standard, was mixed with 600 μl of 0.5 *M* NaOH and transferred to an Extrelut-1 glass column which was preconditioned with 6 ml of dichloromethane the day before. After 10 min, the analytes were eluted under gravity with 5 ml of dichloromethane–isopropyl alcohol (9:1, v/v). The organic phase, with 100 μl of methanolic HCl (25 m*M*) added to prevent nicotine loss, was evaporated to dryness under nitrogen and redissolved in 200 μl of water. The absolute recovery of the extraction ranged between 91 and 96% for nicotine, 90 and

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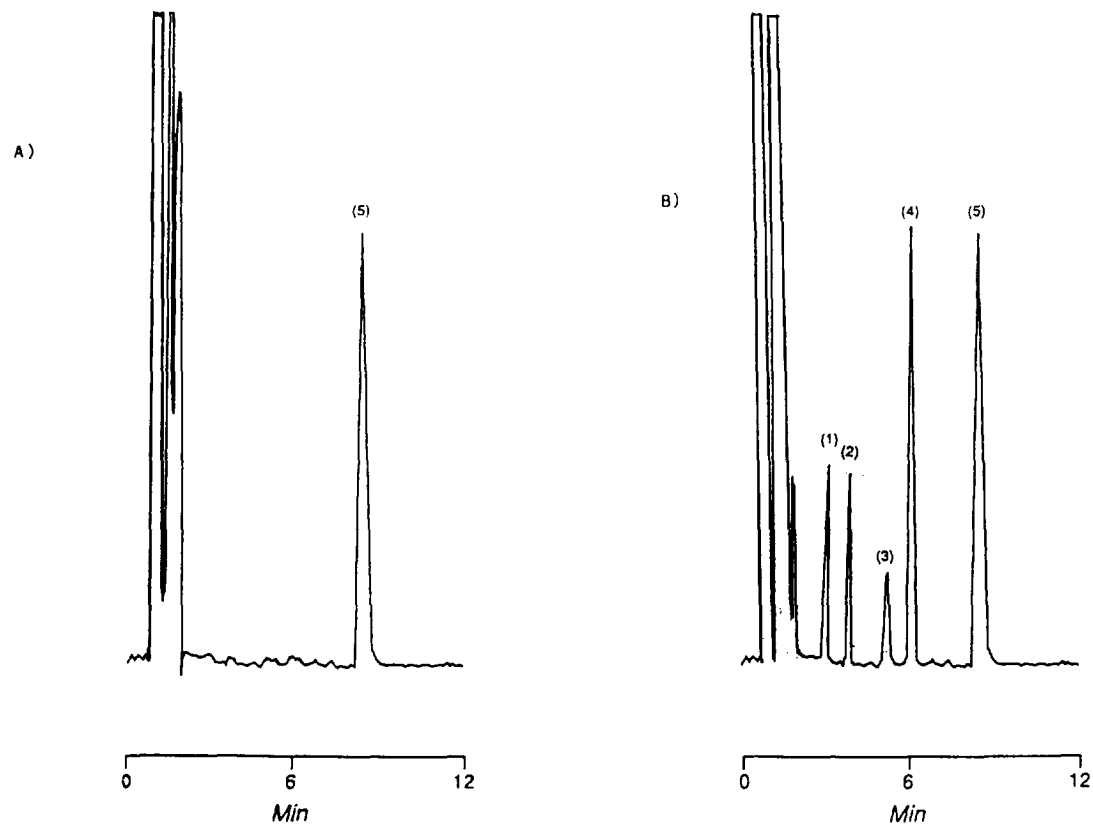

Fig. 1. Chromatograms of (A) extract of a 0.2-ml urine sample from a non-smoker, spiked with 10 μg/ml N-ethylnorcotinine, (B) extract of 0.2 ml urine sample of a smoker containing 1.9 μg/ml *trans*-3′-hydroxycotinine (1), 5.3 μg/ml nicotine (2), 1.2 μg/ml cotinine (3), 5 μg/ml caffeine (4) and 10 μg/ml N-ethylnorcotinine (5).

95% for cotinine, 75 and 80% for *trans*-3′-hydroxycotinine. A typical chromatogram of nicotine, its principal metabolites and caffeine, present in all urine samples, is shown in Fig. 1 and compared to a blank urine sample, obtained from a non-smoker, who consumed caffeine-free drinks. The column used was an LC_8DB steel column (5 μm particle size spherical silica bonded to octylsilane and deactivated for basic compounds, 25 cm × 4.6 mm I.D., Supelchem, Rome, Italy) with a mobile phase consisting of water–acetonitrile (80:9, v/v), containing 5 ml of triethylamine, 670 mg/l sodium heptanesulphonate, and 0.034 *M* each of K_2HPO_4 and citric acid (pH = 4.4), at a flow-rate of 1.6 ml/min. The within-day and between-day coefficients of variation, calculated as described previously [3], were 2.5 and 4% for 100 ng/ml cotinine and 3 and 6% for 100 ng/ml each of nicotine and *trans*-3′-hydroxycotinine in spiked urine samples.

Our findings indicate that solid-phase extraction is a reliable and quick procedure which can also be applied for the determination of other nicotine metabolites, using HPLC conditions described elsewhere [3].

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