ACTA CHROMATOGRAPHICA, NO. 13, 2003

INVESTIGATION OF NICOTINE TRANSFORMATION PRODUCTS BY DENSITOMETRIC TLC AND GC–MS

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SUMMARY

Samples of nicotine were exposed to air and UV light and the products formed were separated by TLC on RP-18 with acetonitrile–water, 88:12 (*v*/*v*), as mobile phase. GC–MS analysis was also performed to confirm the identities of the nicotine transformation products. Cotinine, reported to be one of the main products resulting from the action of environmental agents on nicotine, was identified, as was nicotyrine. The results obtained were compared with nicotine biotransformation products identified in urine from children and pregnant women. *trans*-3′-Hydroxycotinine was identified in these samples but was absent from the products formed by exposure of nicotine to air.

INTRODUCTION

Malignant tumours of the respiratory tract, the upper digestive tract, the bladder, renal pelvis or pancreas have been shown to be causally related to cigarette smoking [1–3]. Nicotine, one of 4000 compounds found in tobacco smoke [4], plays an essential role in the pathogenesis of many diseases, e.g. atherosclerosis, cancer, and neurohormonal changes [4–7]. It is known that cotinine, nicotine oxides, cotinine oxides, and hydroxycotinine are oxygenated and/or hydroxylated products of nicotine metabolism [8–10]. Nicotine, in common with other organic compounds occurring in polluted air, can be transformed to a variety of derivatives under the action of agents such as UV irradiation, oxygen, ozone, oxides of nitrogen and sulphur, and free radicals [8,11]. Both passive and active smokers are, therefore, exposed not only to nicotine, but also to its transformation products. The synergistic effect of air pollution and active smoking on adult pulmonary function was investigated by Xu and Wang [12].

The purpose of this work was to investigate, by thin-layer chromatography (TLC) and gas chromatography coupled with mass spectrometry (GC–MS), products formed from nicotine by the action of environmental agents and to compare the compounds identified with metabolic products of nicotine previously identified in urine.

EXPERIMENTAL

Samples of nicotine standard (50 and 100 μL) were placed on watch glasses and exposed to air and to UV irradiation (λ = 254 nm). After one day and after six weeks the samples were analysed by TLC and GC–MS.

Urine samples from 100 pregnant women and 444 children from the Upper Silesia region in Poland were analysed in accordance with a procedure described elsewhere [13,14].

TLC

TLC separations were performed on 4 cm × 8 cm plates coated with C_{18}-bonded silica gel (Macherey–Nagel, Düren, Germany). Standards (0.5 μg μL^{-1} in acetonitrile) and sample solutions were applied to the plates by means of a Nanomat applicator (Camag, Muttenz, Switzerland). The products from exposure of samples of nicotine were dissolved in dichloromethane (1 mL) and the solutions (1 μL) were applied to the TLC plates. Chromatograms were developed to a distance of 7.5 cm in a horizontal chamber (DSII; Chromdes, Lublin, Poland); the mobile phase was acetonitrile–water, 88:12 (*v*/*v*), containing 1-octanesulfonate sodium (50 mg per 100 mL).

Visualisation was performed, initially, under UV illumination at λ = 254 nm, and then, after densitometric analysis, under visible light after derivatization with Dragendorff's reagent [15]. The spots were quantified by 'zigzag' scanning in reflectance mode (at λ = 260 nm) by means of a CS 9301 PC scanner (Shimadzu, Japan).

GC–MS

GC–MS analysis was performed with a Shimadzu QP 2000 mass spectrometer connected to a GC-14 gas chromatograph (Shimadzu). Standards and samples (1 μL splitless injection) were separated on a 25 m × 0.2 mm i.d. fused silica capillary column coated with a 0.25 μm film of HP Ultra 1. Helium was used as carrier gas; the flow rate was 1 mL min^{-1}. For analysis of nicotine, cotinine, and *trans*-3′-hydroxycotinine

the injector, interface, and ion source temperatures were 250, 270, and 200°C, respectively; the column oven temperature was maintained at 80°C for 2 min after injection then programmed at 6° min^{-1} to 270°C. The mass spectrometer was operated in electron-impact mode (electron energy 70 eV).

RESULTS AND DISCUSSION

Samples of fresh nicotine standard and of the products formed by exposure of nicotine to UV light under environmental conditions were analysed by TLC. The results obtained are shown in Fig. 1. After scanning, the plates were visualised by use of Dragendorff's reagent (Fig. 2), which enables detection of small amounts of nicotine (10 ng per spot). This reagent was also used in previous research to reveal the absence of metabolised nicotine in biological samples [14].

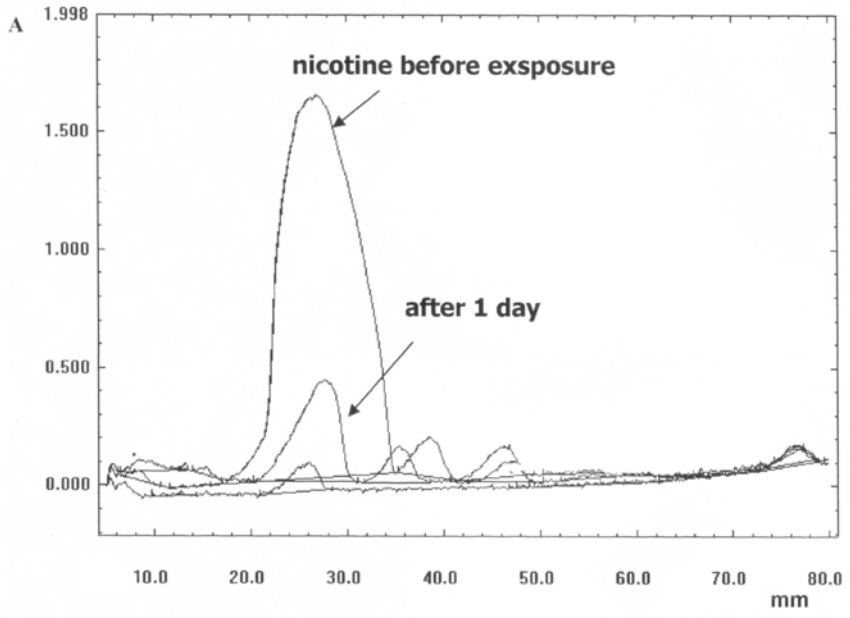

Fig. 1

Comparison of nicotine densitogram with those obtained from the products formed by exposure of nicotine to environmental conditions. Chromatography was performed on RP-18 with acetonitrile–water, 88:12 (*v*/*v*), as mobile phase.

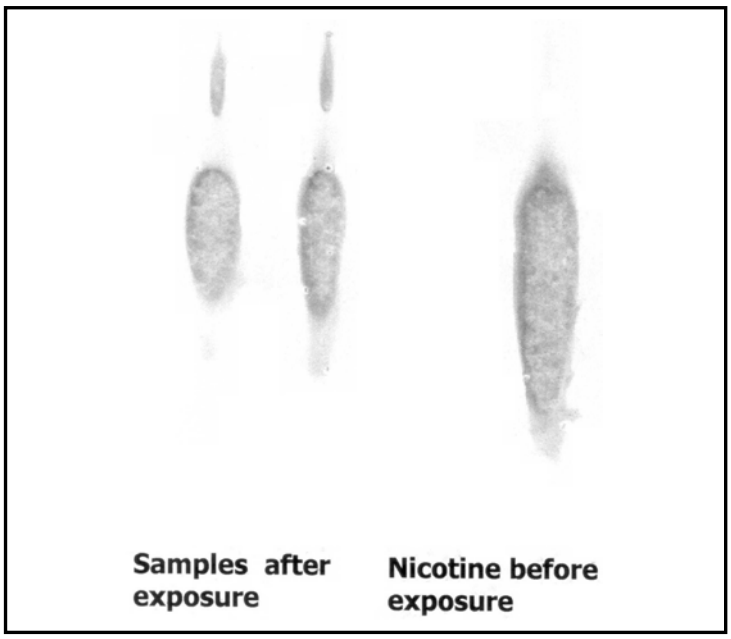

Fig. 2

Visualisation of nicotine and its conversion products on an RP-18 TLC plate after development with acetonitrile–water, 88:12 (*v*/*v*), and reaction with Dragendorff's reagent.

Further analysis of the products formed by exposure of nicotine to environmental conditions was performed by GC–MS. The results from these investigations are shown in Fig. 3. The compounds cotinine and nicotyrine (the mass spectrum of which is presented in Fig. 4) were identified among the products formed by transformation of nicotine.

When similar techniques were used to investigate nicotine biotransformation products, cotinine and *trans*-3′-hydroxycotinine were identified as the main products. These compounds were, for example, identified in the urine of pregnant women by use of TLC with densitometry (Fig. 5). GC–MS was again used for confirmation of these results; mass spectra of the compounds identified are presented in Fig. 5.

CONCLUSIONS

Cotinine has been identified by TLC and GC–MS as the main product formed when nicotine is exposed to environmental conditions. This compound is also found in urine samples from passive and active

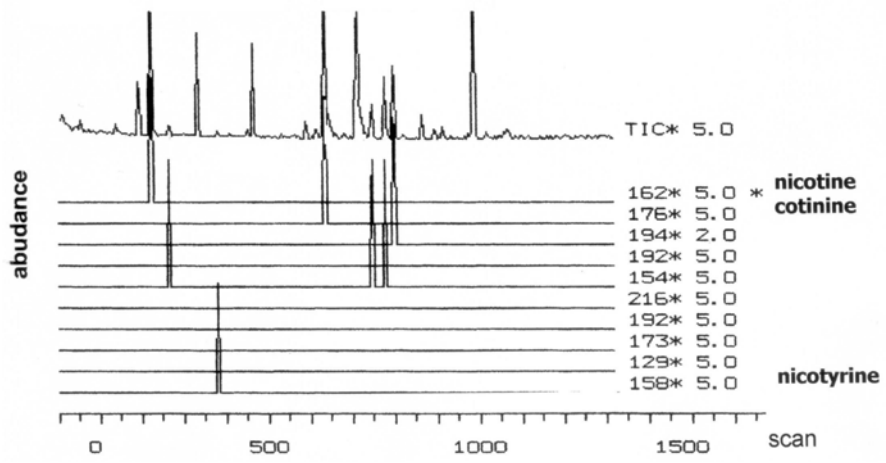

Fig. 3

GC–MS chromatogram obtained from the products formed by exposure of nicotine to environmental conditions.

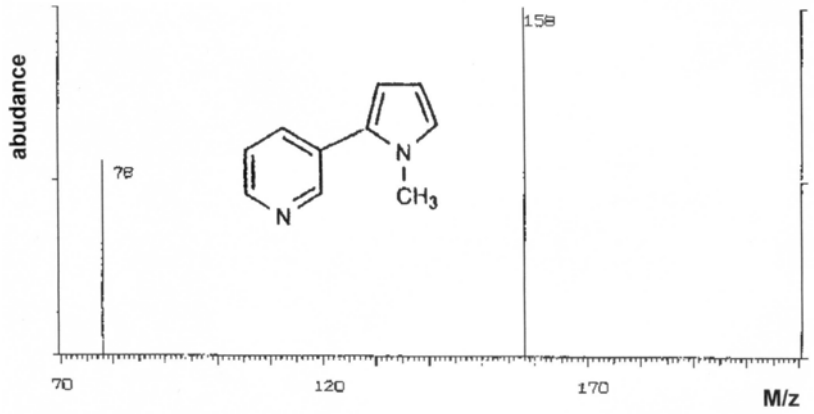

Fig. 4

Mass spectrum of nicotyrine identified among the products formed by exposure of nicotine to environmental conditions.

smokers. Nicotyrine has not previously been identified in urine samples by GC–MS; *trans*-3′-hydroxycotinine has been detected in urine but not among the products formed by exposure of nicotine to environmental conditions. Application of GC–MS enables detection of more compounds formed from nicotine under environmental conditions but because reliable standards

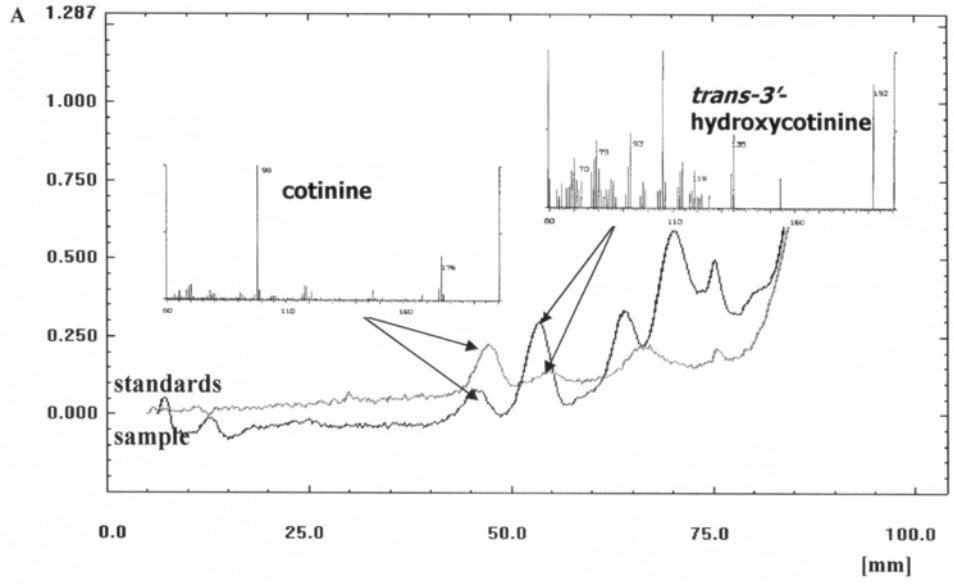

Fig. 5

Densitogram obtained from the fraction isolated from the urine of pregnant women, separated on RP-18 with acetonitrile–water, 88:12 (*v*/*v*), as mobile phase. The main nicotine metabolites and their mass spectra are shown.

were not available unequivocal identification of all the compounds formed was not possible.

TLC with densitometry can be also used for investigation of the purity of standards that decompose readily.

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