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Note

Rapid and sensitive determination of low concentrations of nicotine in plasma by gas chromatography with nitrogen-specific detection

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The determination of nicotine has become increasingly important for the evaluation of new smoking-cessation therapies (transdermal systems). The aim of such treatments is to achieve a sufficient plasma concentration of nicotine to counter-act the craving for cigarettes. To test such systems numerous assay procedures, involving gas chromatography (GC), high-performance liquid chromatography, or gas chromatography–mass spectrometry, for nicotine as well as for its major metabolite cotinine have been published [1–30]. Practically all the procedures reported so far are fairly complicated and not suitable for application to large numbers of samples. The method reported here is based on a procedure published by Feyerabend and Russel [5]. The method was improved in terms of simplicity and thus speed of processing.

A simple, fast and sensitive GC method was therefore developed for the quantitation of unchanged nicotine alone in large numbers of human plasma samples. The method is based on the addition of quinoline as an internal standard, a single-step extraction without solvent evaporation followed by GC with nitrogen-specific detection.

EXPERIMENTAL

Reagents and chemicals

Aqueous solutions were prepared of nicotine base ($C_{10}H_{14}N_2$, MW 162.24) (Serva, Heidelberg, F.R.G.) and quinoline base (C_9H_7N, MW 129.15) (Fluka, Buchs, Switzerland). Laboratory grade 5 *M* NaOH was from Ciba-Geigy (Basle, Switzerland). Toluene and *n*-hexane (Ciba-Geigy) were distilled over a 1-m Vigreux column.

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Procedure

A 1–3 ml volume of plasma, 0.1 ml of internal standard solution (11.6 nmol/ml quinoline in water), 1 ml of 5 *M* NaOH and 0.2 ml of toluene–*n*-hexane (1:1) were shaken for 5 min on a horizontal mechanical shaker at 200 rpm. After brief centrifugation the organic phase was transferred to a small conical vial and aliquots of 5 μl are injected into the GC system.

Gas chromatographic conditions

A Carlo-Erba Vega gas chromatograph equipped with a nitrogen-specific detector was utilized. The chromatograph was fitted with an autosampler Model A200S. This autosampler is capable of handling very small sample volumes, *e.g.* 100 μl.

The column was a 2 m × 4 mm I.D. Pyrex glass column packed with 3% Carbowax 20 M and 1% KOH on Chromosorb W, 80–100 mesh. The flow-rates were 30 ml/min for the carrier gas (helium), 5 ml/min for the hydrogen and 50 ml/min for the air. The temperatures were 147°C for the column oven, 200°C for the injector and 300°C for the detector.

Under these conditions the retention times were 2.25 min for nicotine and 2.75 min for quinoline.

Chromatograms of extracts of blank plasma samples, of plasma samples from a volunteer after application of a transdermal nicotine system and of a sample spiked with nicotine and/or quinoline are given in Fig. 1.

Stability

Stock solutions of nicotine in water are stable for at least two months if stored in the refrigerator at 4°C.

RESULTS AND DISCUSSION

Calibration curves

A calibration curve was prepared by addition of nicotine (31–832 pmol) in aqueous solution to blank plasma samples. After mixing, the samples were processed as described above and chromatographed. The peak-height ratios (nicotine to quinoline) were plotted *versus* the original nicotine concentrations and evaluated by linear least-squares regression analysis ($y = a + bx$). The parameters were as follows: $a = 0.002748$, $b = 0.001735$; $r = 0.9996$; $S_y = 0.01573$; $n = 13$. The calibration graph was linear in the given concentration range.

Recoveries, precision, limit of quantitation and specificity

Aliquots of an aqueous solution of nicotine were added to blank plasma samples to produce concentrations between 37 and 844 pmol/g ($n = 94$). The samples were analysed according to the described method. The found *versus* given concentrations were plotted and evaluated by linear least-squares regression analysis

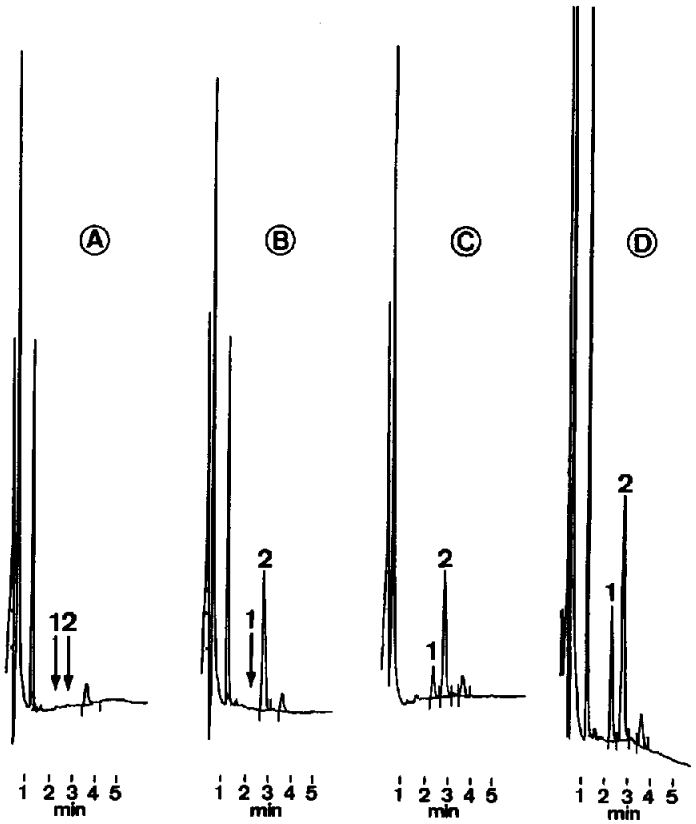

Fig. 1. Chromatograms of extracts of 1-ml plasma samples. (A) Blank plasma sample; (B) sample spiked with 1.16 nmol of quinoline; (C) sample from a volunteer, 8 h after application of a transdermal nicotine system (142 pmol of nicotine and 1.16 nmol of quinoline); (D) sample spiked with 308 pmol of nicotine and 1.16 nmol of quinoline. Peaks: 1 = nicotine; 2 = quinoline (internal standard).

(Fig. 2). The recovery was 97.8 ± 1.7% in the range 138–844 pmol/g and 97.5 ± 16.6% in the range below 137 pmol/g. The coefficient of variation (C.V.) was below 10% in the concentration range 138–844 pmol/g and 17% in the range 37–137 pmol/g (Table I).

The limit of quantitation (LOQ) was estimated ($2 \cdot S_y$, Fig. 2) to be *ca.* 44 pmol per sample (7 ng) and the limit of detection (LOD) *ca.* 12 pmol per sample (2 ng). Based on the fact that up to 3 ml of plasma may be used for the analysis, the method is capable of detecting as little as 4 pmol/g (< 1 ng) of nicotine.

The major metabolite cotinine and the thermally instable nicotine N-oxide do not interfere with the assay of nicotine.

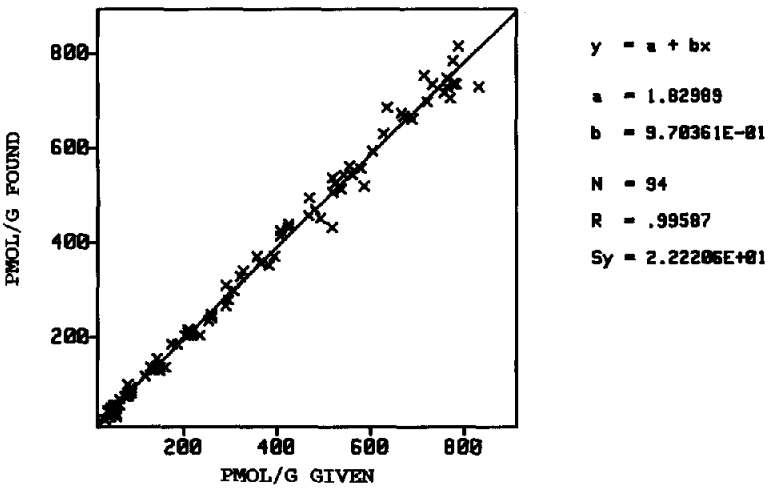

Fig. 2. Method validation. Analysis of plasma samples spiked with nicotine. Concentration range, 37–844 pmol/g; $n = 94$. Linear least-squares regression analysis.

Application

An experimental transdermal nicotine system (area 30 cm^2), delivering 0.7 mg of nicotine per cm^2 over a period of 24 h, was applied to ten healthy volunteers. Blood samples were collected between 0 and 30 h after application. The samples were centrifuged immediately, the plasma transferred to clean tubes and analysed according to the procedure described above.

The mean ± S.D. plasma concentrations of unchanged nicotine are illustrated in Fig. 3.

TABLE I

METHOD VALIDATION: ANALYSIS OF PLASMA SAMPLES SPIKED WITH NICOTINE

Concentration range, 37–844 pmol/g; $n = 94$.

Range (pmol/g)	n	Recovery (%)	C.V. (%)
37–137	24	97.5 ± 16.6	17.0
138–238	18	98.1 ± 6.9	7.0
239–339	10	96.6 ± 5.4	5.6
340–440	10	100.1 ± 4.8	4.8
441–541	9	96.6 ± 6.5	6.7
542–642	8	98.4 ± 5.6	5.7
643–743	6	99.5 ± 3.1	3.1
744–844	9	95.2 ± 4.9	5.1

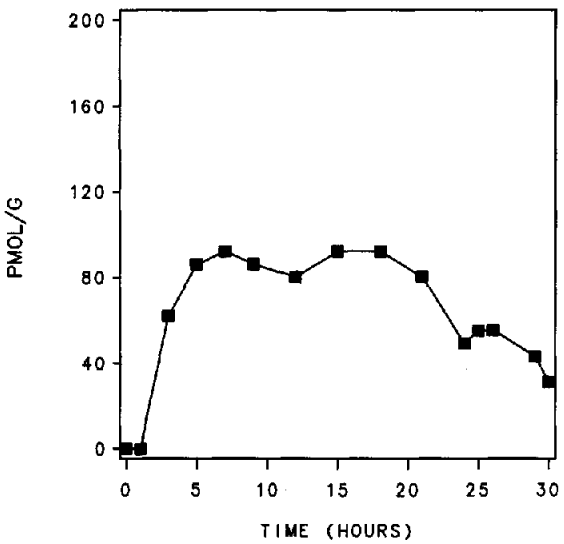

Fig. 3. Plasma concentrations of nicotine in a healthy volunteer wearing a Nicotine TTS (30 cm^2 skin contact area, delivery rate of nicotine 0.7 mg/cm^2 per 24 h) for 24 h.

CONCLUSION

This method is a reliable procedure for the determination of low plasma levels of nicotine after applications of transdermal nicotine systems. The method is, due to its simplicity, suitable for the processing of large numbers of samples.

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