A rapid gas-liquid chromatographic estimation of nicotine in biological fluids

C. FEYERABEND, T. LEVITT AND *M. A. H. RUSSELL

Poisons Unit, New Cross Hospital, London, S.E.14 5ER.
**Addiction Research Unit, Institute of Psychiatry,*
Maudsley Hospital, London, S.E.5 8AF, U.K.

A rapid gas-liquid chromatographic method for the estimation of nicotine in plasma is described. Nicotine is extracted from alkalinized plasma into diethyl ether. This is then concentrated by evaporation and, after an acid back extraction is re-extracted into n-heptane (nitrogen detector) or dichloromethane (flame ionization detector) before injection onto the gas chromatograph. Thirty samples a day can be analysed by this method which enables concentrations of 0·1 ng ml^{-1} nicotine to be measured. It is thus possible to measure nicotine in plasma and urine samples from non-smokers.

Previously published methods for assessing nicotine in body fluids have disadvantages (Schievelbein & Grundke, 1969; Turner, 1969; Burrows, Corp & others, 1971). For the estimation of nicotine in plasma the gas-chromatographic method of Isaac & Rand (1972) requires direct extraction of nicotine with diethyl ether. In our hands, this technique has given poor reproducibility with extraneous peaks on the chromatograms. A more controlled extraction procedure to obviate these difficulties is described below.

MATERIALS AND METHODS

To plasma samples (3 ml) in 12·5 ml glass stoppered tapered centrifuge tubes was added sodium hydroxide (5 N; 2 ml) and diethyl ether (3 ml). After agitation on a Vortex mixer (2 min) the tube was centrigued (10 min) and the organic layer removed to a second tube; this extraction was repeated using a further 3 ml of ether. The bulked extracts were evaporated gently (room temp.) under a stream of nitrogen to 200–500 μl. Hydrochloric acid (2 N; 100 μl) was added and the mixture vortexed (2 min). After centrifugation the ether layer was discarded and the aqueous layer was washed with ether (0·5 ml) which was subsequently discarded. Any ether remaining above the aqueous layer was evaporated under a stream of nitrogen. An aqueous solution of quinoline (0·5 μg ml^{-1}; 100 μl) was added as internal standard and the tube then agitated and centrifuged to ensure adequate mixing.

After the solution had been transferred to a Dreyer tube, sodium hydroxide (5 N; 400 μl) and n-heptane (50 μl) were added. The tube was vortexed for 1 min and then centrifuged. The heptane layer (5 μl) was injected on to the gas chromatograph.

Chromatography

A Hewlett-Packard model 5750 gas-chromatograph equipped with a nitrogen detector (Rubidium bromide crystal; model 15161B) was used. The column was a 6ft × 6 mm (configuration 16) glass tube which had been silanized over 24 h with a

5 % (v/v) solution of dimethyldichlorosilane in toluene. This was packed with 80–100 mesh chromosorb W (Perkin-Elmer Ltd.) coated with 10% w/w Apiezon L and 10% KOH. The instrument settings were as follows: column temperature, 170°; injection port temperature, 210°; detector temperature, 400°; carrier gas (helium) flow-rate, 60 ml min^{-1}; air flow-rate, 300 ml min^{-1}; hydrogen flow-rate, 26 ml min^{-1}. Calibration curves were constructed by adding nicotine to plasma to give concentrations of 5, 10, 20 and 50 ng ml^{-1}. These were then carried through the extraction procedure. The calibration curve of the peak height response was linear over the working range of 1 to 100 ng ml^{-1}. The retention times for quinoline and nicotine were 2·8 and 3·6 min respectively.

Ten determinations were carried out at each of the above concentrations. The results with means and standard deviations are:

ng ml^{-1}	5	10	20	50
Mean (s.d.)	5·28 (0·25)	10·23 (0·67)	19·78 (0·93)	49·76 (1·04)

The absolute recovery of nicotine was estimated by assaying an n-heptane solution containing 10 ng ml^{-1} of nicotine and 50 ng ml^{-1} of quinoline added to plasma where approximately 75% of the added nicotine was recovered.

Sensitivity

This method is sufficiently sensitive to detect plasma nicotine concentrations of 0·1 ng ml^{-1}. The common drugs listed below do not interfere with the analysis.

Imipramine	Fenfluramine	Sodium cromoglycate
Amitriptyline	Ephedrine	Terbutaline
Nortriptyline	Pethidine	Beclomethasone
Adrenaline	Methylamphetamine	Otrivine
Amphetamine	Chlorpheniramine	Salbutamol

DISCUSSION

Isaac & Rand (1972) and Dr. I. E. Burrows (in a personal communication) noted a peak corresponding to nicotine in plasma samples drawn from non-smokers. We also noted this and identified it as nicotine by chromatography with an authentic specimen under three different column conditions. Isaac & Rand (1972) disregarded this peak which they stated was 6 to 17% of the height of their internal standard peak, whilst Horning, Horning & others (1973) reported a content of nicotine in non-smokers of 5% of that in smokers, and found nicotine in laboratory air two days after the room had been vacated.

Samples were assayed by our method in laboratories where smoking was either entirely or partially restricted. Either aqueous or plasma samples assayed in the former conditions gave a constant nicotine level of 0·5 ng ml^{-1}. When the same samples were assayed in the partially restricted atmosphere the levels rose to 4 ng ml^{-1}. We, therefore, suggest that the nicotine from the atmosphere may be absorbed by plasma during the extraction procedure and our results have been corrected each time by subtracting the appropriate "blank" of 0·5 ng ml^{-1}.

Samples taken from non-smokers who had deliberately avoided a smoking environment for at least 12 h gave nicotine concentrations of 0·5 ng ml^{-1} and therefore true values of 0. But when the same volunteers were re-examined after their usual degree
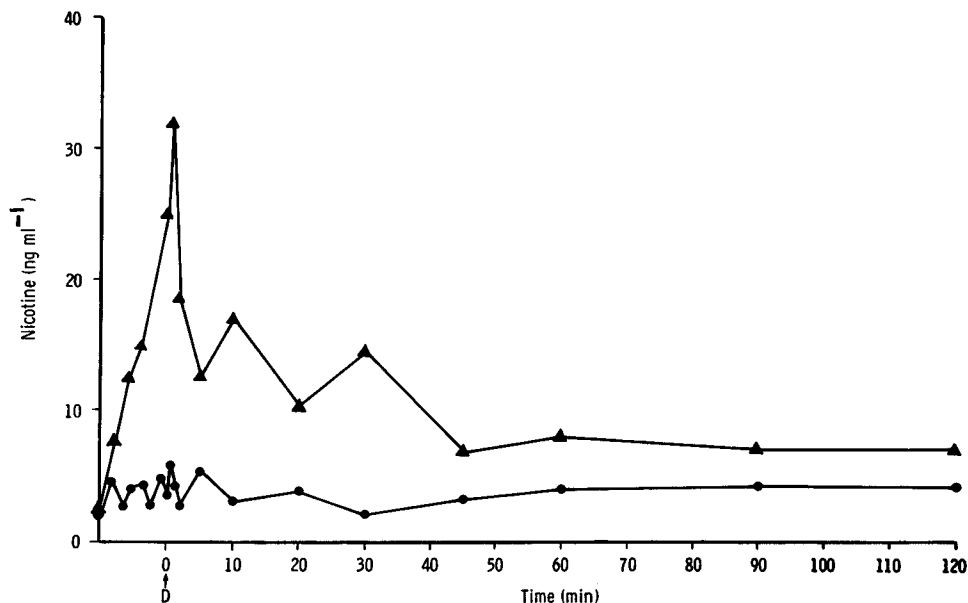
FIG. 1. Plasma nicotine concentrations in an inhaling smoker (▲) and a non-inhaling smoker (●) during and after smoking one cigarette which was discarded at the point D.

of exposure to "smokey atmospheres" their corrected plasma nicotine concentrations ranged from 0·1 to as high as 6·8 ng ml⁻¹. It is invalid therefore to correct plasma nicotine concentrations obtained in smokers by subtracting plasma "blank" values derived from non-smokers.

Fig. 1 illustrates typical venous plasma nicotine profiles obtained using the method described. In the inhaling smoker, the plasma nicotine concentrations rose steadily throughout the duration of smoking reaching a peak of approximately 30 ng ml⁻¹ shortly after the cigarette had been discarded. The concentrations then fell over the next 60 min to a resting level of approximately 7 ng ml⁻¹. By contrast, the non-inhaling smoker maintained concentrations throughout of between 2 and 5 ng ml⁻¹. It is of interest to note that these "resting" levels were similar to those we have encountered in non-smokers working in areas adjacent to smokers.

Acknowledgement

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