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Note

Capillary-column combined gas chromatography–mass spectrometry method for the estimation of nicotine in plasma by selective ion monitoring

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The gas chromatographic (GC) determination of nicotine in plasma has been carried out previously using a nitrogen-selective flame ionization detector (NFID)[1,2]. These methods, although sensitive, are not entirely specific for nicotine. GC peaks, corresponding in retention time to nicotine, were obtained from the plasma of non-smokers[1,2]. Using mass spectrometry (MS), Horning *et al.*[3] were able positively to identify nicotine in the urine of non-smokers, and these authors concluded that non-smokers absorb nicotine (via the lungs) from room air.

GC–MS was used to confirm the identity of nicotine extracted from whole blood, in the development of a GC method for the estimation of nicotine[4]. However, this GC–MS method required 100 ml of whole blood, and quantitation of nicotine was not carried out. A preliminary account of a GC–MS method for the quantitative determination of nicotine in plasma, using selective ion monitoring (SIM), was described briefly by Pilotti *et al.*[5], who used a GC column packed with 8% Carbowax 20M (2% KOH) on Gas-Chrom Q (100–120 mesh), and deuterated nicotine as internal standard.

In the present method, good resolution, sensitivity and specificity were obtained by attaching a glass capillary column directly to a mass spectrometer, which was operated in the SIM mode. Nicotine was extracted from plasma by a modification of the method of Fayerabend *et al.*[2], which allowed the direct addition of the internal standard to plasma prior to extraction. Quinoline used as internal standard in the NFID–GC method[2], was found to be a convenient internal standard in the present method.

Glassware

Glassware was cleaned by immersion overnight in a detergent (Pyroneg) solution, rinsed with water, ethanol and distilled water[4], then dried in an oven in a laboratory where smoking was strictly prohibited.

EXPERIMENTAL

Extraction of nicotine from plasma

An aqueous solution of quinoline (5 μg/ml; 100 μl) was added as internal

standard to plasma (3 ml) in a 12.5-ml glass-stoppered centrifuge tube, followed by sodium hydroxide (5 *N*; 2 ml) and diethyl ether (3 ml). The tube was agitated on a vortex mixer (1 min) and then shaken for 10 min on a mechanical shaker. The organic layer was recovered (after centrifuging) and extraction of the aqueous layer was repeated using a further 3 ml of ether. The combined ether extracts were evaporated in a stream of nitrogen, and concentrated to 200–500 μl. Hydrochloric acid (2 *N*; 200 μl) was added and the mixture was agitated for two min, centrifuged, and the ether removed. The aqueous layer was washed with ether (0.5 ml), the ether removed, and any remaining on top was blown off in a stream of nitrogen. The aqueous solution was transferred to a dryer tube, sodium hydroxide (5 *N*; 400 μl) was added and the tube was agitated by vortex mixer. Benzene (100 μl) was added and the tube was agitated, centrifuged, and 0.8 μl of the benzene layer was injected into the GC–MS.

Gas chromatography–mass spectrometry

A Varian 1400 gas chromatograph equipped with a capillary injector (split ratio 1:10) and a glass capillary column (20 m × 0.3 mm I.D.) coated with SP1000 (Supelco, Bellefonte, Pa., U.S.A.) was used. The column temperature was 160°, and the helium flow-rate 0.5 ml/min.

A V.G. (Altrincham, England) Micromass 12B mass spectrometer was directly coupled via a 0.15 mm I.D. glass capillary restriction to the gas chromatograph. Operating conditions for the mass spectrometer were: interface, 250°; ion source, 200°; ionising potential, 70 eV; accelerating voltage, 4 kV; and source pressure, 10^{-5} torr.

Only the base peak of the nicotine spectrum (*m/e* 84) and the molecular ion of quinoline (*m/e* 129) were monitored (SIM). The accelerating voltage was switched between 2.6 and 4 kV to bring these ions into focus.

A Rikadenki B-36 pen recorder was used to record the *m/e* 84 and *m/e* 129 signals at 20–50 mV and 200 mV respectively. The chart speed was 200 mm/h.

RESULTS AND DISCUSSION

Mass spectra of nicotine and quinoline

The mass spectra of nicotine (a) and quinoline (b) are illustrated in Fig. 1. The base peak of the nicotine spectrum (*m/e* 84), produced by α-cleavage of the α-substituted pyrrolidine with the loss of pyridine[6], was sufficiently abundant to be measured quantitatively by SIM over the required range of nicotine concentrations. The molecular ion of quinoline (*m/e* 129), which is also the base peak, was sufficiently abundant and close enough in mass to the base peak of nicotine for quinoline to be used as the internal standard.

Sensitivity

The mass chromatograms of nicotine standards along with their respective quinoline internal standard, used to construct a calibration curve, are shown in Fig. 2. Nicotine was added to plasma, in duplicate, to give 100, 75, 50 and 25 ng/ml final concentrations, and extracted together with a plasma blank as described above. The lower limit of detection of nicotine was below 5 ng/ml.

To prolong filament life, and lengthen the time between cleanings, the power

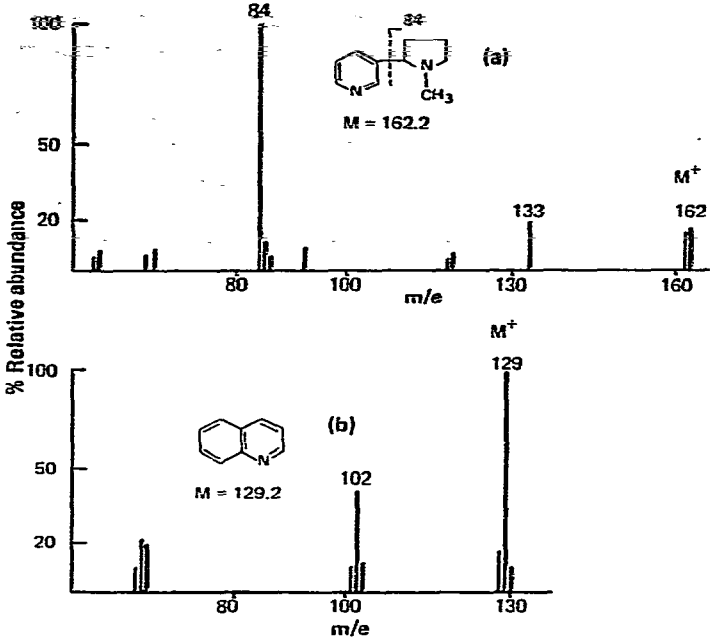

Fig. 1. Electron impact mass spectra of (a) nicotine and (b) quinoline. Only ions with relative abundance > 5% have been plotted.

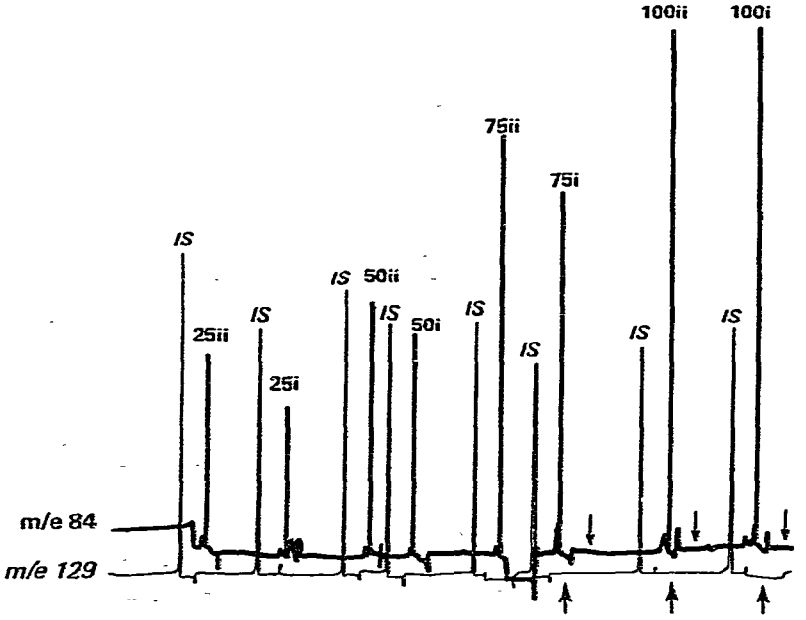

Fig. 2. Mass chromatograms of duplicate (i and ii) nicotine standards and their respective quinoline internal standards (*IS*) extracted from plasma. The arrows indicate the point of injection for the first three samples, and the recorder pens are offset by the distance between the top and bottom arrow. The retention times for nicotine and quinoline were 2.4 and 3 min, respectively.

supply to the source was switched off after each injection, and reset after the solvent peak had been observed on the vacuum gauge. No solvent peaks therefore appear in the chromatograms (Fig. 2).

The calibration curve (Fig. 3) was constructed by plotting the ratio of the peak heights of the *m/e* 84 ion of nicotine, and the *m/e* 129 ion of quinoline (from the chromatograms in Fig. 2) against the concentration of nicotine (ng/ml). This plot is linear over the measured concentration range and was also shown to be linear over the concentration range 5–100 ng nicotine/ml. The line does not pass through the origin as nicotine is detectable in the plasma blank. The concentration of nicotine in plasma was calculated from: (peak height ratio—intercept)/gradient.

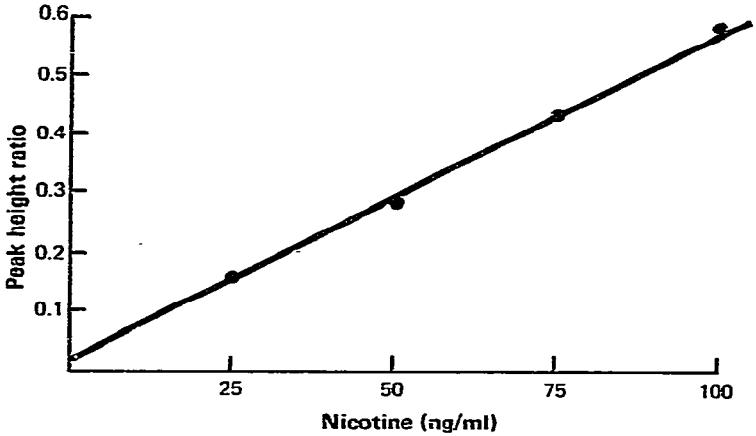

Fig. 3. Calibration curve of duplicate nicotine standards extracted from plasma. The peak height ratio of the *m/e* 84 and *m/e* 129 ions is plotted against concentration of nicotine. This follows the equation for a straight line, with a slope of 0.005, an ordinate intercept of 0.016, and a correlation coefficient of 0.99.

Reproducibility

Nicotine was added to plasma in quadruplet, to give solutions containing 50 and 10 ng/ml. After extraction, the means and standard deviations were (51.9 $\pm$ 2.4) and (10.6 $\pm$ 0.8) respectively. This compares favourably with values of (49.76 $\pm$ 1.04) and (10.23 $\pm$ 0.67) for ten determinations on 50 and 10 ng/ml standards obtained by Fayerabend *et al.*[2] using NFID–GC.

Specificity

Although SIM is not completely specific for a particular molecule, SIM combined with capillary-column GC–MS has a greater specificity due to the high resolution of capillary columns.

The minor tobacco alkaloid anabasine is a potential interfering substance as it also has a base peak at *m/e* 84[6], but since it has double the retention time of nicotine, when analysed on a glass capillary column similar to that used in the present study[5], it is unlikely to interfere.

Measurement of nicotine elimination from plasma

A plot of the decline in plasma nicotine concentration against time is shown in

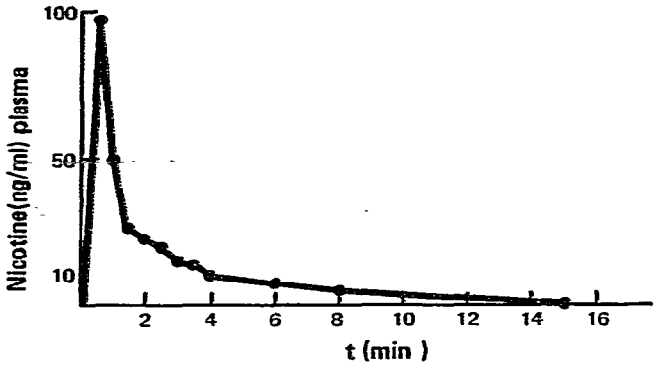

Fig. 4. Decline in plasma nicotine concentration against time in the dog. 10 μg nicotine/kg body weight was injected intravenously, and blood samples taken at the indicated times.

Fig. 4. Nicotine was measured by the present method, which is currently being applied to studies of nicotine elimination in man and experimental animals.

CONCLUSIONS

Nicotine has been estimated in plasma using capillary column GC–MS. This has produced a sensitive and specific method, without the need for deuterated nicotine as internal standard. Although only a small volume (< 0.1 μl) of sample is actually injected into the GC–MS, the sensitivity of the method is enhanced by the sharpness of the mass chromatogram peaks produced by the glass capillary column.

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