Letters to the Editor

Improvements in a Method to Connect an Implement with a Tire Slashing

Sir:

In a previously published article ("Connecting a Knife or Ice Pick to a Tire in a Tire Slashing," Vol. 25, No. 3, July 1980, pp. 603-611), our method for connecting a knife blade or implement to a tire slashing has one portion in the procedure that is difficult to accomplish—the transferring of the particles from the implement to the gradient column—and one portion that is tedious—watching the particle while waiting for it to come to rest in the gradient. The following improvements are proposed to alleviate these problems.

The previously used jeweler's forceps or microcapillary tube has been eliminated in favor of a differently shaped buret to contain the gradient column. A 500-mL separatory funnel with the stopcock section and part of the top removed is joined to the top of a regular buret. The top portion of the separatory funnel is cut away above the bulge to allow easier access to the gradient (Fig. 1, left). A normal gradient is made in the buret portion of this new column as before. The remainder of the column and the bell portion are filled with distilled water to within 25 mm (1 in.) of the top. The suspect blade is carefully inserted into the column below the water line to prevent accumulation of air bubbles on the blade and attached rubber particles. A vibrating tool, such as an engraving marker, is then gently touched to the blade to knock the particles into the column. The shape of the bell at the top channels the particles down into the buret where they are spotted with the meniscus magnifier as before. The top portion of the bell allows a small amount of splashing without the loss of the particles. This method is quicker and takes less practice in transferring particles than using a jeweler's forceps or microcapillary tube. In addition, many more particles can be transferred within a very short time.

After the particles have been transferred to the gradient column it takes between 3 and 30

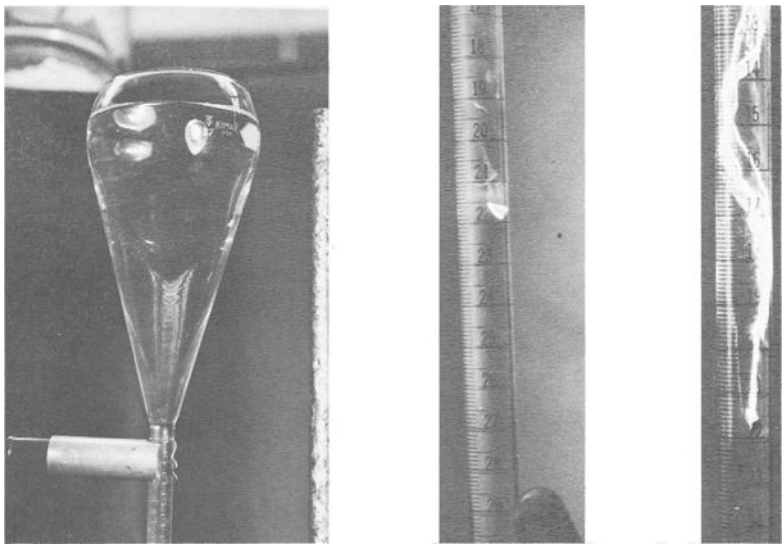

FIG. 1—(Left) *Separatory funnel.* (Middle) *Large particle in the gradient under UV light.* (Right) *Fluorescent trail left by porous ink.*

reported nicotine and cotinine plasma levels determined either by radioimmunoassay [*8*] or by GC nitrogen selective detection [*4-7,9,10*].

Materials and Instrumentation

Gas chromatography combined with nitrogen selective detection was performed on a Perkin-Elmer GC, Model 900, equipped with a rubidium-bead nitrogen-phosphorus detector. Signal output was recorded on a 1-mV Varian recorder, Model A-25. A siliconized glass column (1.83 m by 2 mm inside diameter) packed with 3% SE-30 on 100-120 Gas Chrom Q was used for analysis. Injector and manifold temperatures were 250 and 300°C, respectively. Column temperature was programmed at a rate of 24°C/min from 150 to 200°C. The initial temperature was held for 3 min and the final temperature held for 4 min. Helium carrier gas, air, and hydrogen flow rates were 30, 120, and 3 mL/min, respectively.

Nicotine was purchased from Sigma Chemical Co. (St. Louis, Mo.). Cotinine was obtained as a gift from Dr. Edward R. Bowman, Medical College of Virginia (Richmond, Va.). Ketamine hydrochloride (CI-581) was a gift from Warner-Lambert/Park Davis Pharmaceutical Research Division (Detroit, Mich.). All solvents were pesticide grade.

Biological material was obtained from adult volunteer smokers who were provided a study protocol and signed an advised consent form before entering the study. Approximately 16 mL of blood was collected from the subjects into heparinized tubes. The blood samples were centrifuged at 3500 rpm in an International Centrifuge, Model K, and the plasma was transferred into stoppered glass tubes and frozen at −20°C until analysis.

Methods

Throughout the procedure, extractions were carried out in a 15-mL siliconized conical glass centrifuge tube fitted with a Teflon®-lined screw cap. Organic and aqueous phases were separated by centrifugation. A methanolic solution of the internal standard ketamine hydrochloride (6 mg/L) was prepared and 25 μL added to 1 mL plasma. The plasma was made basic (pH > 11) by the addition of one drop of an aqueous 10% potassium hydroxide solution; 5 mL of methylene chloride was added; the solution was agitated for 10 min on a mechanical shaker; and the phases were separated. The aqueous phase was aspirated and the organic phase decanted into a clean tube. One millilitre of 0.2*M* hydrochloric acid was added to the organic phase and the mixture vortexed for 30 s. The phases were separated and the organic layer was removed with a transfer pipet. The remaining aqueous phase was made basic by the addition of two or three drops of potassium hydroxide solution; 5 mL methylene chloride was added, and the mixture was vortexed for 30 s. The phases were separated and the aqueous phase was discarded. The organic phase was dried briefly over approximately 300 mg anhydrous sodium sulfate and decanted into a clean tube, and then 2 μL of a solution of 1 g/L oxalic acid in methanol was added. The mixture was vortexed and the methylene chloride removed by evaporation at 50°C under a stream of nitrogen gas. The residue was dissolved in 30 μL of methanol that contained 0.05% *tert*-butylamine, and 1 to 2 μL was injected into the GC.

Quantitation of nicotine and cotinine was based on a least squares plot of peak height ratio of nicotine or cotinine to the internal standard versus the concentration of nicotine (5 to 25 μg/L) or cotinine (50 to 250 μg/L). Standard solutions of nicotine and cotinine were prepared daily and determined along with each daily analysis of unknowns.

Results and Discussion

The relationship between peak height and plasma concentration of nicotine and cotinine was linear over the range of 5 to 25 μg/L and 50 to 250 μg/L, respectively (correlation r^2 =

0.98, both cases). Standard curves for nicotine and cotinine based on triplicate analyses are shown in Figs. 1 and 2. The linearity, though not shown, was experimentally verified to extend to 50 and 500 μg/L for nicotine and cotinine, respectively. The deviation about the mean of each data point is indicated by the vertical bars, and the average percentage of deviation for all points is the mean coefficient of variation (*CV*) for the method.

The method mean *CV*s are 14.3 and 9.3% for nicotine and cotinine, respectively, and are comparable to those reported by Hengen and Hengen [*4*] for the extraction of nicotine and cotinine. There is one notable difference in the cotinine standards used in the two methods. The cotinine standard we obtained was crystalline and has a melting point of 52°C, nearly identical to that first described by Beilstein [*11*], whereas the cotinine standard used by Hengen and Hengen was a distilled material [*4*].

The method was sensitive to 2 ng nicotine and 5 ng cotinine per millilitre extracted. The sensitivity for detection of nicotine is about 1/20th less than that reported by Feyerabend and Russell [*7*] and Hengen and Hengen [*4*] using a nitrogen detector (different column packing) but about the same per millilitre as that reported by Isaac and Rand [*6*] using a nitrogen detector (different column packing). Recovery is not considered a factor in this difference. Seventy and 95% nicotine and cotinine, respectively, were recoverable by the extraction. We found no improvement in detector response to nicotine with a 3% SP 2250-DB packing as compared with a silicone 3% SE-30 packing, although Hengen and Hengen did [*4*]. We believe the difference is instrumental.

We observed that the alkali detector did not respond linearly to less than 150 pg nicotine injected. This "threshold" response gave rise to a curve that did not pass through the origin (Fig. 1). Similar observations with regard to a threshold response and a standard curve not passing through the origin were reported by Isaac and Rand [*6*] for the quantitation of nicotine with an alkali flame detector. Of the various GC nitrogen selective detection methods reported for the quantitation of nicotine in plasma, the lowest workable calibration standard appears to be 5 to 10 μg/L [*4–7*]. The present method is no exception.

Nicotine and cotinine levels were determined in 150 plasma samples. The results of the analyses are summarized in Table 1. Typical chromatograms of smokers' plasma (Panel A) and an extracted aqueous standard (Panel B) of 20, 200, and 150 μg/L nicotine, cotinine, and ketamine hydrochloride, respectively, are shown in Fig. 3. The use of blank human plasma for the preparation of standards was avoided since it is apparently quite difficult to obtain completely nicotine-free plasma [*4–7*]. Calibration standards were prepared in purified water (Milli-Q-System, Milliport Corp., Bedford, Mass.) that had no interfering or background peaks.

The extraneous peak at 4.2 min (Fig. 3) was a contaminant in both the "disposal biotips" (Schwaz/Mann, Becton Dickinson Co., Orangeburg, N.Y.) and "micropets" (Clay Adams, Becton Dickinson Co., Parsippany, N.J.) that were used initially to pipet microlitre-sized

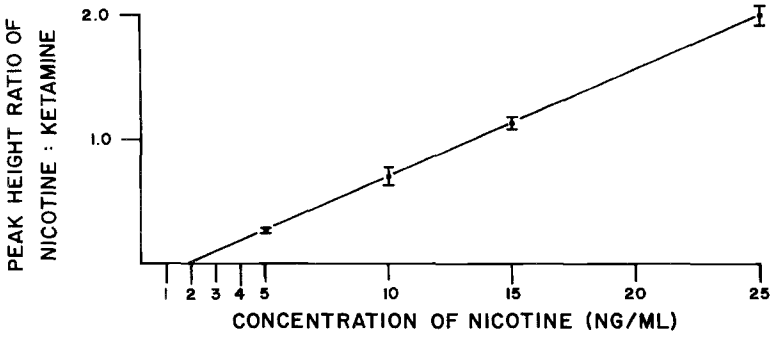

FIG. 1—*Standard curve for nicotine from aqueous extractions.*

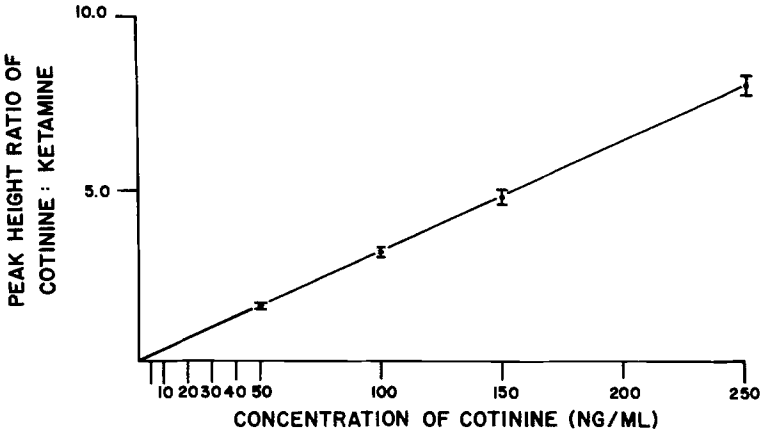

FIG. 2—*Standard curve for cotinine from aqueous extractions.*

TABLE 1—*Mean nicotine and cotinine plasma levels*[a] *of 150 samples obtained from habitual cigarette*[b] *smokers.*

	Nicotine, ng/mL	Cotinine, ng/mL
Mean	19.5	219
Standard error	±1	±13
Range	3-63	20-260

[a]Blood drawn approximately 2 min after smoking.

[b]Nicotine content, 0.1 to 1.4 mg per cigarette.

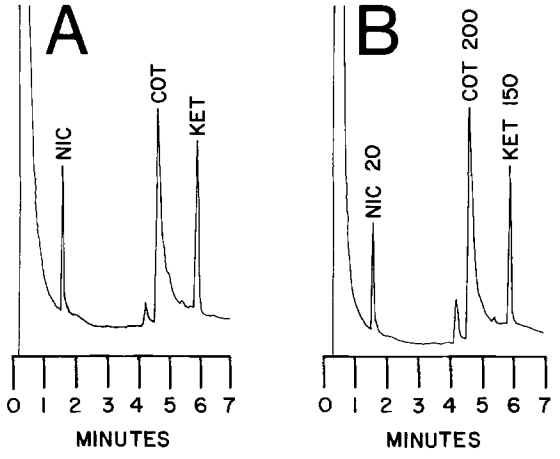

FIG. 3—(A) *Chromatogram of a habitual smoker's plasma extract.* (B) *Chromatogram of extracted standard from an aqueous solution containing 20, 200, and 150 ng/mL nicotine (NIC), cotinine (COT), and ketamine hydrochloride (KET), respectively.*

volumes of standards and solvents. The level of this nitrogenous contaminant could be as much as 10 to 15 times larger than shown. At high levels it interfered with the quantitation of nicotine, presumably by competing for the oxalic acid that was added to prevent loss of nicotine during evaporation of the solvent. Discontinuing the use of these disposable pipets eliminated the problem completely.

All samples in which nicotine was detected also contained cotinine. The range of cotinine plasma levels shown in Table 1 coincides with the range of 73 to 650 μg/L cotinine measured in approximately 240 smokers by Langone et al [*8*]. The mean cotinine plasma level of 219 μg/L reported in this communication is close to the value of 150 to 260 μg/L measured in two habitual smokers [*8*]. The 3 to 63 μg/L range of nicotine plasma levels reported in this study falls within the range of approximately 70 [*8–10*] to 10 μg/L [*6*] that has been reported for habitual smokers. The mean nicotine plasma level of 19.5 μg/L we reported is in agreement with the plasma profile of an inhaling smoker reported by Feyerabend et al [*5*]. Their study indicated that, within the first 5 min after smoking, nicotine plasma levels fell from approximately 32 to 12 μg/L while at 2 min after smoking, the time closest to blood sampling in our subjects, the levels declined to approximately 19 μg/L [*5*].

We found it convenient to do the extractions a day before the chromatographic analysis and to store the dried extract at 7°C overnight. The extracts in some instances gave rise to a peak that eluted at approximately 15 min when the column temperature was held at 200°C. This peak might be another oxidative metabolite of nicotine. It is doubtful that it is cotinine *N*-oxide, a metabolite isolated in monkey urine [*12*]. A single extraction method would not favor its removal from plasma, and the high injector and column temperatures most likely would result in the loss of oxygen and conversion to cotinine [*12*].

In conclusion, this method provided simultaneous measurement of nanogram levels of nicotine and cotinine. Use of the method allows the forensic science analyst to quantitate, relative to one internal standard, both components from a single chromatographic report.

Acknowledgment

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