Sensitive detection of nicotine after its novel perfluoroacylation and analysis using capillary gas chromatography–electron-capture detection

James M. Moore*, Donald A. Cooper, Theodore C. Kram and Robert F.X. Klein

Special Testing and Research Laboratory, US Drug Enforcement Administration, 7704 Old Springhouse Road, McLean, VA 22102-3494 (USA)

ABSTRACT

The on-column chromatographic detection of nicotine at low picogram levels is described. Nicotine is first subjected to chemical derivatization with heptafluorobutyric anhydride in the presence of pyridine. In the absence of pyridine, the derivatization reaction is markedly retarded. This high-yield reaction results in the opening of the N-methylpyrrolidine ring of nicotine with concomitant formation of a highly electrophilic N,O-diheptafluorobutyryl derivative. After extraction of the nicotine derivative into isooctane, it is subjected to splitless capillary gas chromatographic analysis using a ^{63}Ni electron-capture detector and a moderately polar fused-silica capillary column. The nicotine derivative can be detected on-column at levels below 5 pg.

INTRODUCTION

Nicotine, **1**, an N-methylpyrrolidine-substituted pyridine alkaloid of *Nicotiana Tabacum*, has been the subject of numerous studies. We have reviewed 36 selected references that describe the analyses of **1** in a variety of matrices [1–36]; most address the detection of **1** and/or its metabolites in biological specimens, including plasma [4,6–13,16,17,20,23,24,26,28,30,35,36], blood [1,3,13], urine [5,7,13,16,19–21,24–27, 29,30,32,34], saliva [7,11] and tissue [15]. Similar methods have been applied for the detection of **1** in tobacco [14], allergenic extracts of tobacco [18], environmental samples [9,31,33] and neat [2,22]. Due to recent concerns over the passive inhalation of tobacco smoke, detection of environmental contamination by **1** has come into sharp focus [31,33]. Of the various methods reviewed, all but one [25] were chromatographic, with twenty-one utilizing gas chromatography (GC) [1–4,6–13,15–17,20,23,24,30,31,35,36] and thirteen employing high-performance liquid chromatography (HPLC) [5,14,18,19,21,22,26–29,32–34]. Of the GC methods reviewed, twelve utilized nitrogen–phosphorous detection [4,7,9–13,15,17,23,30,31], six employed mass spectrometry (MS) [6,15,20,30,35,36], three utilized flame ionization detection [1,3,16] and three employed electron-capture detection (ECD) [2,8,24]. Six of the HPLC procedures relied upon UV detection [5,14,18,19,21,33], while radiometric detection [26], electrochemical detection [22,28] and MS [34] were used in four methods. Finally, in three instances HPLC analyses were preceded by chemical derivatization (ChD) to give colored products which were subsequently measured in the visible range [27,29,32].

In general, GC analysis of **1** and its congeners was superior to HPLC in terms of on-column detectability and specificity. Typical GC on-column minimum detectable quantity (OC-

---

* Corresponding author.

MDQ) levels were usually in the 50–100 pg range, with a few methods reporting lower than 10 pg [8,9,13,17] and one method below 100 fg [36]. The highest specificities were observed with high-resolution capillary columns and element-specific or, especially, MS detectors. In contrast to GC, typical OC-MDQs for HPLC ranged from 100 pg to over 10 ng. However, the overall methodology MDQ (MTH-MDQ) was still reasonably sensitive due to the much larger HPLC injection volumes. In addition, HPLC was better suited for the analysis of certain highly polar metabolites of 1 (e.g., nicotine N-oxide), which either do not chromatograph or exhibit poor chromatographic behavior using GC.

Our interest in 1 has been peripheral and evolved during investigations into the detection of coca alkaloids in refined, illicit cocaine samples, in particular the N-methylpyrrolidine alkaloids hygrine and cuscohygrine. Specifically, we were interested in determining whether either alkaloid underwent high-yield heptafluorobutyrylation at a carbon site for subsequent analysis using capillary gas chromatography-electron-capture detection (cGC–ECD). We have previously reported such unusual acylation/derivatization reactions for the cGC–ECD analyses of fentanyl [37], morphine N-oxide and didehydroheroin [38]. Since hygrine and cuscohygrine were not commercially available, the N-methylpyrrolidine-substituted alkaloid nicotine, 1, was utilized as a model compound.

Herein, we report the successful derivatization of 1 with heptafluorobutyric anhydride (HFBA) in the presence of pyridine. The resultant derivative exhibited excellent chromatographic behavior and was extremely sensitive towards cGC–ECD analysis, with an OC-MDQ below 5 pg. The overall methodology represented a significant improvement over most existing procedures in terms of on-column detectability.

EXPERIMENTAL

Standards, solvents and chemicals

Nicotine, 1 (98%), was obtained from the Aldrich (Milwaukee, WI, USA). Other drug standards were acquired from the standards collection of the Special Testing and Research Laboratory, US Drug Enforcement Administration (McLean, VA, USA). All solvents except water were distilled-in-glass products of Burdick and Jackson (Muskegon, MI, USA). Aldrin (utilized as an internal standard) was obtained from Supelco (Bellefonte, PA, USA). HFBA, supplied in 1-ml ampules, was obtained from Pierce (Rockford, IL, USA). All other chemicals were reagent grade.

Glassware

All derivatization reactions and extractions were performed in either 5- or 15-ml glass-stoppered centrifuge tubes. Prior to use, the tubes were treated with hot Nochromix (commercial oxidant)–sulfuric acid solution, rinsed with water, methanol and acetone and dried under vacuum.

Capillary gas chromatography–electron-capture detection

All chromatograms were generated in the splitless mode using two Hewlett-Packard Model 5880 gas chromatographs interfaced with Hewlett-Packard Level IV data processors; both were equipped with ^{63}Ni electron-capture detectors (15 mCi). For the initial phase of this study, gas chromatograph No. 1 was fitted with a 15 m × 0.25 mm I.D. fused-silica capillary column coated with DB-5 + (0.25 μm) (J&W Scientific, Rancho Cordova, CA, USA). The oven temperature was multilevel programmed as follows: (level 1) initial temperature, 90°C, initial hold, 1.0 min; temperature program rate, 25°C/min; final temperature, 200°C; final hold, 1.0 min; (level 2) temperature program rate, 4.0°C/min; final temperature, 275°C; final hold, 10 min. In the latter part of this investigation, the 15-m DB-5 + column was replaced by a 30 m × 0.25 mm I.D. column coated with DB-5 (0.25 μm). Gas chromatograph No. 2 was fitted with two separate 30 m × 0.25 mm I.D. fused-silica capillary columns coated with DB-1 (0.25 μm) and DB-1701 (0.25 μm), respectively. For all three 30-m columns, the oven temperatures were multilevel programmed as follows: (level 1) initial temperature, 90°C, initial hold, 1.5 min; temperature program rate, 25°C/min; final tem-

perature, 170°C; final hold, 1.0 min; (level 2) temperature program rate, 4.0°C/min; final temperature, 275°C; final hold, 30 min. Injector and detector temperatures were maintained at 200 and 300°C, respectively. Hydrogen (Zero Grade, Roberts Oxygen, Merrifield, VA, USA) was used as the carrier gas at a linear velocity of between 35–45 cm/s and measured for isooctane at an oven temperature of 90°C. An argon–methane (95:5) mixture (Roberts Oxygen) was the make-up gas at a flow-rate of between 30 and 40 ml/min. During the splitless injection, the solvent was vented after a 1.0-min hold. Chart speeds and attenuation settings are given in the appropriate figures.

Capillary gas chromatography–mass spectrometry

Low-resolution electron ionization (EI) and negative chemical ionization (NCI) mass spectra were acquired on a Finnigan-MAT Model 4630 (San Jose, CA, USA) quadrupole mass spectrometer. In the initial phase of this study, the cGC–MS was fitted with a 15 m × 0.25 mm I.D. fused-silica capillary column coated with either 0.25 μm DB-1 or DB-5. In the latter part of this investigation, a 15 m × 0.25 mm I.D. fused-silica capillary column coated with 0.25 μm RT$_x$-200-Methyl Trifluoropropyl (Restek, Bellefonte, PA, USA) was used. Sample injection was accomplished with an on-column injector (J&W Scientific) at a helium carrier velocity of 60 cm/s for methane. All EI data were acquired at an ionization potential of 60 eV at a source temperature of 120°C (uncorrected). All NCI data were acquired via cGC introduction of the sample; methane was utilized as the reagent gas. The source temperature was 80°C (uncorrected).

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy

All NMR spectra were obtained in deuterated chloroform on a Bruker AM-30 WB NMR spectrometer operating at 300.1 MHz for ^{1}H and 75.5 MHz for ^{13}C. Tetramethylsilane was utilized as an internal calibration standard. Gated decoupled ^{13}C, ^{1}H–^{1}H COSY and ^{1}H–^{13}C HETERO-COSY experiments were run using standard Bruker software.

Infrared spectroscopy

A Beckman 4240 infrared spectrometer and KBr plates were used in the recording of all infrared (IR) spectra.

Standard solutions

Solution A. An anhydrous acetonitrile solution of **1** at a concentration of 0.10 mg/ml.

Solution B. An aliquot of solution A diluted with anhydrous acetonitrile to a concentration for **1** of 25 ng/ml.

Solution C. An anhydrous acetonitrile solution of **1** (0.10 mg/ml), ephedrine (0.10 mg/ml), methamphetamine (0.20 mg/ml), and tetracaine (0.20 mg/ml).

Derivatization procedures

Microgram level. To 1.0 ml of standard solution A or C in a 15-ml glass-stoppered centrifuge tube was added 50 μl of HFBA. After vortexing, 100 μl of pyridine was added. After again vortexing, the solution was heated at 75°C for 15 min. After cooling, 8.0 ml of isooctane (containing aldrin at 100 pg/μl) and 5 ml of saturated aqueous sodium bicarbonate were added. The tube was immediately shaken vigorously for 5–10 s and then centrifuged at 2000 rpm (796 g) for 5 min. The isooctane extract was isolated and dried over anhydrous sodium sulfate. A 1.0 ml aliquot of the isooctane extract was diluted to an appropriate volume with additional isooctane (containing aldrin internal standard at 100 pg/μl). About 2 μl of the final isooctane extract was injected into the cGC–ECD under conditions described under Experimental.

Nanogram level. For determination of MTH-MDQ, 200 μl of standard solution B was dispensed into a 5-ml glass-stoppered centrifuge tube. To the tube was added 10 μl of a solution containing 10% (v/v) HFBA in anhydrous acetonitrile followed by 10 μl of a solution containing 20% (v/v) pyridine in acetonitrile. After vortexing, the solution was heated at 75°C for 75 min. After cooling, 2–3 ml of petroleum ether (containing aldrin internal standard at 100 pg/μl) and 1 ml of saturated aqueous sodium bicarbonate were added. The tube was immediately shaken vigorously for 5–10 s and then centrifuged. The petroleum ether extract was passed

through anhydrous sodium sulfate into another tube and evaporated just to dryness (55–65°C/ N_2). The residue was reconstituted in 100 μl of ethyl acetate at about 75°C for 2 min, and about 2 μl of the resulting solution was injected into the cGC–ECD under the conditions described in the Experimental section.

RESULTS AND DISCUSSION

Nicotine derivatization reaction and structural elucidation of product

Reaction of 1 with HFBA in the presence of pyridine resulted in scission of the C2'–N bond and addition of heptafluorobutyryl (HFB) and heptafluorobutyryloxy [HFB(O)] moieties to the molecule, giving 2. This reaction is illustrated in Fig. 1. The ring cleavage was highly unusual and in sharp contrast with our previously reported HFBA derivatization of nitrogen heterocycles, wherein the nitrogen-containing ring remained intact and the reaction resulted in formation of a ring-substituted HFB-vinylogous amide with HFB attachment at a carbon site [37,38]. We have additionally observed that when the N-
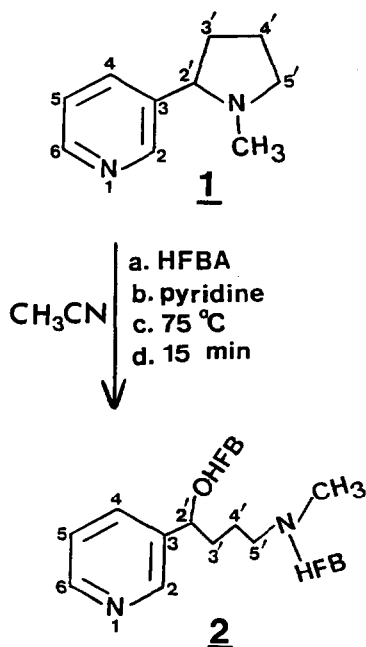
methylpyrrolidine-containing compound hygrinol, obtained by lithium aluminum hydride reduction of hygrine, was subjected to similar HFBA derivatization, the N-methylpyrrolidine ring also remained unopened, with HFB attachment at a carbon site [39]. These results strongly suggested that the presence of the aromatic ring at the α carbon in nicotine directed the HFBA derivatization reaction towards an alternate, previously unknown reaction pathway (Fig. 1).

Structural confirmation of the nicotine derivatization product was accomplished as follows. Milligram quantities of 2 were prepared and subjected to cGC–MS, ^{1}H and ^{13}C NMR and IR analyses.

GC–MS. The mass spectrum of the perfluorinated nicotine derivative, illustrated in Fig. 2, was acquired using three different capillary columns, coated with DB-1, DB-5 and RT_x-200; all were found to be similar. The EI spectrum of 2 displayed an $M^{+\cdot}$ at $m/z = 572$, which was confirmed by an intense $M^{-\cdot}$ at $m/z = 572$ under negative chemical ionization conditions. This molecular mass corresponds to incorporation of an HFB and an HFB(O) group into 1 with concomitant opening of the N-methylpyrrolidine ring. Fragment ions in the EI spectrum occurring at $m/z = 553$, 375 (base peak) and 359 are probably due to losses of F, HFB and HFB(O), respectively. Cleavage at C2'–C3' and C3'–C4' is believed to give rise to ions at $m/z = 304$ and 318, respectively, with charge retention on the pyridinyl-containing moiety. An intense fragment ion at $m/z = 240$ is probably due to fission of the C4'–C5' bond with charge retention on the HFB-amide moiety.

In order to confirm the presence of an HFB(O) substituent, 2 was subjected to hydrolysis with warm methanol. After removal of the solvent, the residue was divided into two equal aliquots, with one-half being treated with N,O-bis(trimethylsilyl)acetamide (BSA) and the other half treated with [²H₉]BSA, the deuterated analogue of BSA. The EI-MS spectrum of the resultant trimethylsilyl (TMS) derivative yielded the expected molecule ion at $m/z = 448$, which shifted to $m/z = 457$ as the [²H₉]TMS derivative, suggesting the new derivative to be N-HFB-O-TMS-nicotine. The base peak fragment ion at

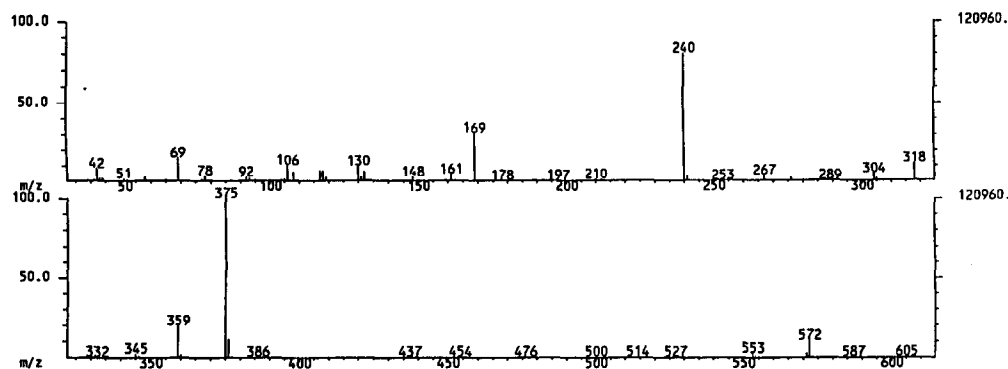Fig. 1. Derivatization of nicotine with heptafluorobutyric anhydride in the presence of pyridine.

Fig. 2. Electron ionization mass spectrum of N,O-di-HFB-nicotine. Column: 15 m × 0.25 mm I.D. Rt$_x$-200.

$m/z = 180$ for this derivative supported an assignment of the HFB(O) group at C2'. This ion probably arises from cleavage of C2'–C3' bond with charge retention on the pyridinyl-CH$_2$-OTMS moiety. As expected, the ion at $m/z = 180$ is shifted 9 u for the [^{2}H$_9$]TMS derivative.

^1H and ^{13}C NMR. One- and two-dimensional NMR analyses, confirmed the ring-opened structure of 2, as seen in Table I. The ^{1}H and ^{13}C resonances of the pyridinyl ring substituent were only slightly perturbed from 1 [40]. In the aliphatic chain, the protons displayed complex multiplets at 6.0(1H), 3.5(2H), 1.9(2H), and 1.7(2H) ppm, assigned as C$_{2'}$–H$_1$, C$_{5'}$–H$_2$, C$_{3'}$–H$_2$ and C$_{4'}$–H$_2$, respectively, on the basis of ^{1}H–^{1}H COSY. The N-methyl group displayed 2 singlets (3.1 and 2.9 ppm) in a 3:1 ratio, due to

amide resonance [41]. The aliphatic chain and N-methyl carbons were assigned on the basis of ^{1}H–^{13}C HETEROCOSY. The perfluorinated carbons were grouped as a highly complex series of multiplets from 100 to 120 ppm. The amidic carbonyl showed a triplet at 157.5 ppm and the ester carbonyl a triplet at 171.0 ppm.

IR. 2 was subjected to IR analysis as an oil between KBr salt plates. The spectrum was dominated by 2 carbonyl bands, at 1780 cm^{-1} [HFB(0) ester] and 1680 cm^{-1} (N-HFB amide). This data compares favorably with the N-HFB derivative of pyrrolidine and the N,O-di-HFB derivative of ring-opened N-methylpyrrolidine.

Nicotine derivatization yield

The yield of 2 at the μg level was >85%. This

TABLE I

^{1}H AND ^{13}C NUCLEAR MAGNETIC RESONANCE DATA FOR N,O-DI-HFB-NICOTINE

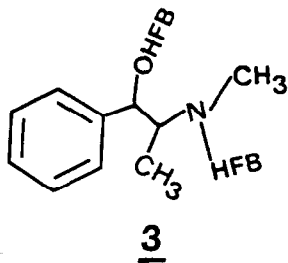| Proton	δ	Multiplicity	Carbon	δ	Multiplicity
C2-H	8.6	br m	C2	149.8	d
			C3	134.2(?)	s
C4-H	7.7	d	C4	134.5	d
C5-H	7.2	dd	C5	123.9	d
C6-H	8.6	br m	C6	147.5	d
C2'-H$_2$	6.0	m	C2'	78.1	d
C3'-H$_2$	1.9	m	C3'	32.4	t
C4'-H$_2$	1.7	m	C4'	22.0	t
C5'-H$_2$	3.5	m	C5'	48.8	t
CH$_3$	3.1/	d-s	CH$_3$	34.6	q
	2.9				

Fig. 3. Perfluorinated derivative of ephedrine.

was verified against an internal standard prepared by the derivatization of an equal amount of ephedrine, which also yielded an N,O-di-HFB derivative, **3**, seen in Fig. 3. Fig. 4 illustrates the cGC–ECD chromatogram of N-HFB-methamphetamine, **3**, **2**, aldrin internal standard and N-HFB-tetracaine on the 15-m DB-5 + column. The comparable responses for **2** and **3** at the 1-ng

level on-column is apparent. The reduced response for the methamphetamine and tetracaine derivatives is due to the fact that both contain only a single HFB group.

Derivative yield versus reaction temperature and time variables

The reaction yield of **2** was studied at 25, 75 and 95°C and at times from 1 min to 2 h. Significant yields were realized at 25°C after as little as 1 min (at the 100 μg level). Modest improvements in yields were noted after 15 min at 75 or 95°C. The results at 75 and 95°C were comparable and no further improvement was noted at 75°C even after extending the reaction from 15 min to 2 h.

Reaction by-products or GC injection port artifacts

During cGC–ECD analyses of **1** on the 15-m DB-5 + column, two later-eluting minor peaks (A and B) were observed. In order to improve their resolution, the 15-m DB-5 + column was replaced with a 30-m DB-5 column. When **1** was derivatized at 75°C for only 5 min, the intensity of A was quite significant, whereas B was undetectable. When the reaction was extended to 75 min, A was markedly diminished and B appeared, as illustrated in Fig. 5. Under either conditions, the yield of **2** was high and virtually unvaried.
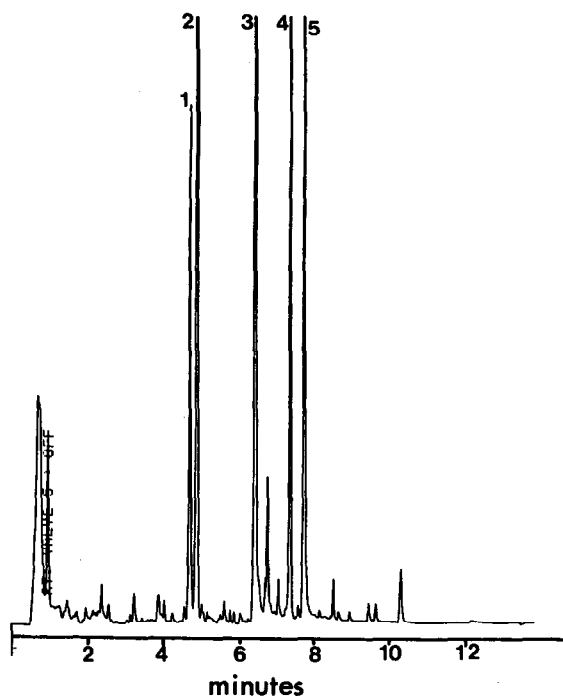

Fig. 4. Capillary gas chromatographic–electron capture detection chromatogram of perfluorinated nicotine and other drug derivatives. Column: 15 m × 0.25 mm DB-5 + ; splitless injection; attenuation: 2[8]. Peaks: 1 = N-HFB-methamphetamine, 2 ng; 2 = N,O-di-HFB-ephedrine, 1 ng; 3 = N,O-di-HFB-nicotine, 1 ng; 4 = Aldrin internal standard, 200 pg; 5 = HFB-tetracaine, 2 ng.
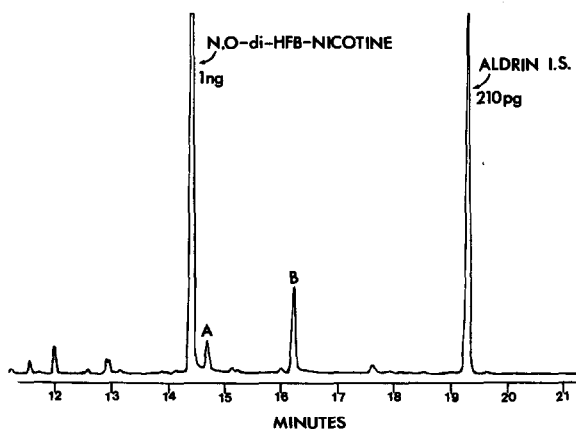

Fig. 5. Capillary gas chromatographic–electron capture detection chromatogram of perfluorinated nicotine and splitless injection port artifacts A and B. Column: 30 m × 0.25 mm DB-5; attenuation 2[8].
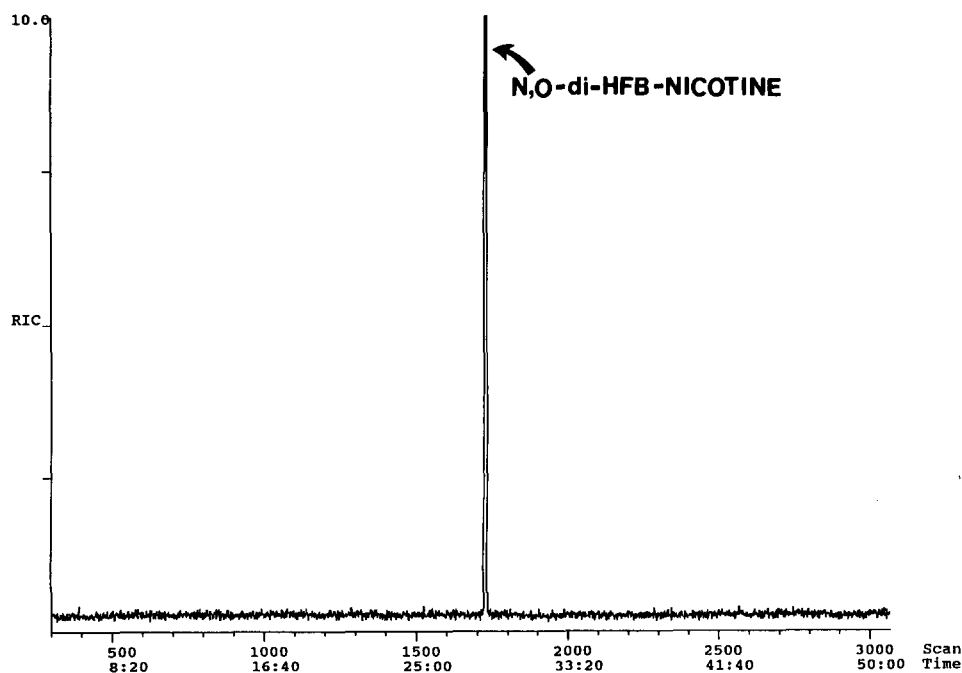
Fig. 6. Capillary gas chromatographic–mass spectrometric chromatogram of perfluorinated nicotine derivative. Column: 25 m × 0.25 mm RT_x-200; on-column injection, 90°C.

The appearance of A and B was also measured as a function of the splitless injection port temperature. It was observed that in going from an injection port temperature of 225 to 300°C, the response for 2 and B decreased, whereas A increased. Furthermore, as seen in Fig. 6, when 2 was injected on-column at 90°C and under pristine conditions into the 15-m Rt_x-200 column, A and B were not detected. These results suggested that A and B were artifacts, a consequence of the splitless injection at elevated temperatures. To maximize response for 2 and minimize artifacts, a splitless injection port temperature range of 200–250°C is recommended.

Derivatization in the presence and absence of added base

The reaction was studied in the presence of pyridine, 4-dimethylaminopyridine (PDAP) and without added base. Maximum yield was realized in the presence of pyridine. When PDAP (a hypernucleophilic acylation catalyst) was used, the yield was substantially reduced. This was surprising, as previous use of PDAP had consistently resulted in high-yield heptafluoro-butyrylation at N, O and C sites in a variety of similar compounds [36–38]. It appears that, in the presence of PDAP, the C2' carbon exists as a carbanion stabilized by the close proximity of the electron withdrawing pyridinyl moiety, thereby inhibiting nucleophilic attack by the [HFB(O)] anion.

Finally, the reaction proceeded without any added base. However, this variant required extended heating times and resulted in substantially reduced yields. The positive reaction in the absence of base was probably due to 1 acting as a self-catalyst, since 1 contains a pyridinyl group.

Chromatography of nicotine derivative

The GC characteristics of 2 were evaluated on all three 30-m columns, *i.e.*, DB-1, DB-5 and DB-1701, and the 15-m Rt_x-200 column. Of the DB columns investigated, optimum chromatography for 2 and highest resolution of A and B were best achieved on the DB-5 column. Perhaps ideal chromatographic behavior was realized on the Rt_x-200 column (Fig. 6). The retention data for 2, A, B and the aldrin internal

TABLE II

CAPILLARY GAS CHROMATOGRAPHY–ELECTRON CAPTURE DETECTION RETENTION TIME DATA FOR N,O-DI-HFB-NICOTINE, INJECTION PORT ARTIFACTS A AND B, AND ALDRIN INTERNAL STANDARD USING 30 m × 0.25 mm DB-5, DB-1701, AND DB-1 CAPILLARY COLUMNS

Column	Retention times (min)			
	N,O-di-HFB-Nicotine	Peak A	Peak B	Aldrin I.S.
DB-5	14.36	14.66	16.15	19.18
DB-1701	14.80	a	17.19	15.89
DB-1[b]	9.96	9.85	10.95	13.59

[a] Artifact peak A coelutes with N,O-di-HFB-nicotine peak.
[b] Initial oven temperature hold time was 4.0 min as compared to 5.0 min for the DB-5 and DB-1701 columns.

standard on the three 30-m columns are summarized in Table II.

On-column and method minimum detectable quantities

The OC-MDQ of 1 (as the di-HFB derivative 2) was determined on the 30-m DB-5 column. About 100 μg of 1 was derivatized as previously described, except at 25°C for 75 min. After isooctane extraction, serial dilutions with additional isooctane were made to achieve a concentration for 1 of about 2.5 pg/μl. After a method blank was run, it was determined that the OC-MDQ of 1 was below 5 pg. Under optimized conditions, such as a shorter column and thinner film (*e.g.*, 0.10 μm), on-column detection at the femtogram level may be within reach.

The procedure for MTH-MDQ has been previously described. About 5 ng of 1 was derivatized using reduced amounts of acetonitrile, HFBA and pyridine in order to reduce artifact contribution. Petroleum ether was used as a replacement extraction solvent, in order to facilitate the subsequent evaporation step. Ethyl acetate was the choice as a reconstitution solvent because of its polarity, moderate boiling point and compatibility with the cGC–ECD system. It was observed that for the 30-m DB-5 column, 5 ng of 1 was easily detected at a nominal instrumental attenuation of 2^7.

Environmental contamination

A frequently encountered problem in ultra-trace level analyses of 1 is environmental con-tamination, *i.e.*, detection of 1 in methodology blanks. Typical contamination sources include air, water and glassware. These problems usually occur in those methods that do not employ ChD (which includes most methods). Environmental contamination is significantly diminished when using the present ChD methodology, because once the ChD reaction has been completed and the excess reagent quenched, any subsequent environmental contamination is undetectable, as the ECD detector responds only to 2 and not 1. The absence of 1 in a method blank was established using this method.

CONCLUSIONS

The derivatization of nicotine with HFBA in the presence of pyridine yields a di-HFB substituted product that exhibits good chromatography and excellent sensitivity when using capillary gas chromatography–electron-capture detection. With an on-column MDQ level of below 5 pg, the method described herein provides one of the most sensitive determinative steps for the detection of nicotine.

REFERENCES

1 I.E. Burrows, P.J. Corp, G.C. Jackson and B.F.J. Page, *Analyst*, 96 (1971) 81.
2 L. Neelakantan and H.B. Kostenbauder, *Anal. Chem.*, 46 (1974) 452.
3 S.E. Falkman, I.E. Burrows, R.A. Lundgren and B.F.J. Page, *Analyst*, 100 (1975) 99.

4 C. Feyerabend, T. Levitt and M.A.H. Russell, *J. Pharm. Pharmacol.*, 27 (1975) 434.
5 I.D. Watson, *J. Chromatogr.*, 143 (1977) 203.
6 J. Dow and K. Hall, *J. Chromatogr.*, 153 (1978) 521.
7 C. Feyerabend and M.A.H. Russell, *J. Pharm. Pharmacol.*, 31 (1979) 73.
8 P. Hartvig, N-O. Ahnfelt, M. Hammarlund and J. Vessman, *J. Chromatogr.*, 173 (1979) 127.
9 O. Grubner, M.W. First and G.L. Huber, *Anal. Chem.*, 52 (1980) 1755.
10 C. Feyerabend and M.A.H. Russell, *J. Pharm. Pharmacol.*, 32 (1980) 178.
11 C. Feyerabend and M.A.H. Russell, *Analyst.*, 105 (1980) 998.
12 M.J. Kogan, K. Verebey, J.H. Jaffee and S.J. Mule, *J. Forensic Sci.*, 26 (1981) 6.
13 P. Jacob, III, M. Wilson and N.L. Benowitz, *J. Chromatogr.*, 222 (1981) 61.
14 J.A. Saunders and D.E. Blume, *J. Chromatogr.*, 205 (1981) 147.
15 J.A. Thompson, M-S. Ho and D.R. Petersen, *J. Chromatogr.*, 231 (1982) 53.
16 G. Stehlik, J. Kainzbauer, H. Tausch and O. Richter, *J. Chromatogr.*, 232 (1982) 295.
17 M. Curvall, E. Kazemi-Vala and C.R. Enzell, *J. Chromatogr.*, 232 (1982) 283.
18 B.J.L. Sudan, C. Brouillard, C. Strehler, H. Strub, J. Sterboul and J. Sainte-Laudy, *J. Chromatogr.*, 288 (1984) 415.
19 K.C. Cundy and P.A. Crooks, *J. Chromatogr.*, 306 (1984) 291.
20 P. Daenens, L. Laruelle, K. Callewaert, P. de Schepper, R. Galeazzi and J. van Rossum, *J. Chromatogr.*, 342 (1985) 79.
21 M.J. Horstmann, *J. Chromatogr.*, 344 (1985) 391.
22 S. Mousa, G.R. van Loon, A.A. Houdi and P.A. Crooks, *J. Chromatogr.*, 347 (1985) 405.
23 R.A. Davis, *J. Chromatogr. Sci.*, 24 (1986) 134.

24 G.B. Neurath and F.G. Pein, *J. Chromatogr.*, 415 (1987) 400.
25 C.L. Smith and M. Cooke, *Analyst.*, 112 (1987) 1515.
26 G.A. Kyerematen, L.H. Taylor, J.D. deBethizy and E.S. Vesell, *J. Chromatogr.*, 419 (1987) 191.
27 R.D. Barlow, P.A. Thompson and R.B. Stone, *J. Chromatogr.*, 419 (1987) 375.
28 C-Y. Chien, J.N. Diana and P.A. Crooks, *LC-GC.*, 6 (1988) 53.
29 M.T. Parviainen and R.D. Barlow, *J. Chromatogr.*, 431 (1988) 216.
30 P. Voncken, G. Schepers and K-H. Schäfer, *J. Chromatogr.*, 479 (1989) 410.
31 M.W. Ogden, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 1002.
32 S. O'Doherty, M. Cooke and D.J. Roberts, *J. High Resolut. Chromatogr.*, 13 (1990) 74.
33 L.J. Lewis, J.D. Lamb, D.J. Eatough, L.D. Hansen and E.A. Lewis, *J. Chromatogr. Sci.*, 28 (1990) 200.
34 K.T. McManus, J.D. deBethizy, D.A. Garteiz, G.A. Kyerematen and E.S. Vesell, *J. Chromatogr. Sci.*, 28 (1990) 510.
35 P. Jacob, III, L. Yu, M. Wilson and N.L. Benowitz, *Bio. Mass Spectr.*, 20 (1991) 247.
36 D. Jones, M. Curvall, L. Abrahamsson, E. Kazemi-Vala and C. Enzell, *Biomed. Mass Spectrom.*, 9 (1982) 539.
37 J.M. Moore, A.C. Allen, D.A. Cooper and S. Carr, *Anal. Chem.*, 58 (1986) 1656.
38 J.M. Moore, A.C. Allen and D.A. Cooper, *Anal. Chem.*, 56 (1984) 642.
39 J.M. Moore, D.A. Cooper and R.F.X. Klein, unpublished results.
40 M. Shamma and D.M. Hindenlang, *Carbon*-13 *NMR Shift Assignments of Amines and Alkaloids*, Plenum Press, New York, 1979, p. 24.
41 R.M. Silverstein, G.C. Bassler and T.C. Marill, *Spectrometric Identification of Organic Compounds*, Wiley, New York, 4th ed., 1981, p. 197.