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Fast Analysis of Nicotine in Tobacco Using Double-Shot Pyrolysis−Gas Chromatography−Mass Spectrometry

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A simple, rapid, and sensitive assay of nicotine in tobacco has been developed using pyrolysis−gas chromatography−mass spectrometry (DSP-GC-MS). The optimum pyrolyzer (desorption) conditions, using Korean tobacco grade B1O (0.4 mg) and *n*-heptadecane as an internal standard, were found to be heating from 50 to 300 °C at a rate of 60 °C/min. Replicate determinations ($n = 5$), on the same tobacco and using *n*-heptadecane as the internal standard, resulted in a nicotine content of 1.96%, with a relative standard deviation (RSD) of 1.9%. This result was in good agreement with those from established methods: the Cai ether extraction method, a chloroform extraction method, and the CORESTA recommended method. However, the DSP method requires less than half the time of the solvent extraction methods, requires less sample, is almost solvent-free, and is less labor-intensive. The DSP method was used to determine the nicotine content of eight flue-cured tobaccos from Brazil, China, Korea, and the United States, which were found to have contents ranging from 1.21 to 2.19%.

KEYWORDS: Nicotine; tobacco; desorption; double-shot pyrolyzer; GC-MS; solvent extraction

INTRODUCTION

Tobacco alkaloids are unique in character as compared with other tobacco constituents, and it is well-known that they significantly affect the taste of tobacco (*1*). Nicotine is generally synthesized in roots, but other alkaloids are synthesized above ground in the stems and leaves (*2*). Among the more than 20 alkaloids found in tobacco, nicotine has the highest levels (*3*) and represents approximately 95% of total tobacco alkaloids (*4*).

There have been several analytical approaches to the analysis of nicotine and minor alkaloids in tobacco, including the following methods: steam distillation spectrophotometry (*5*), micellar electrokinetic capillary chromatography (*6*), solvent extraction−gas chromatography (GC) (*7*−*10*), pyrolysis−GC (*11*), solid-phase microextraction (SPME)−GC (*12*, *13*), and high-performance capillary electrophoresis (*14*).

Among these, the GC methods have been most widely applied to the analysis of nicotine, using flame ionization (*7*, *10*), nitrogen-phosphorus (*8*), or mass selective detectors (*9*, *12*, *13*). Although some of the most popular methods for GC analysis of nicotine and other minor alkaloids involve solvent extraction, using chloroform, diethyl ether, hexane, or methanol, some reports claim that extraction efficiency varies according to solvent (*5*, *15*, *16*).

In 1979, Rosa (*11*) reported a pyrolysis-GC method for the estimation of nicotine and nornicotine in tobacco, using a Victoreen pyrolyzer. The pyrolyzer conditions used in this method were fixed temperature (250 °C). In addition, this method did not use an internal standard for reduction of errors in the quantitative analysis. Furthermore, in Rosa's report, there were about 20% differences in nicotine values determined by the Victoreen pyrolyzer method and the Bush solvent extraction method (*7*).

In this paper, we describe a new nicotine analysis method using the recently developed double-shot pyrolyzer (DSP, Frontier Lab., model 2020iD, Japan) that combines the advantages of thermal desorption and flash pyrolysis. The DSP is temperature-programmable, from ambient temperature to 350 °C and above, in increments ranging from 1 to 60 °C/min. Temperature programming can be used to minimize the formation of thermal decomposition products as the nicotine is desorbed from the tobacco leaves: Indeed, it has been observed previously that thermal desorption at fixed temperatures of 250 °C and above results in significant decomposition (*17*).

Using the method developed in this study, a quantitative analysis of nicotine in flue-cured Korean tobacco (grade B1O) was performed and the results were compared with those from

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the previously reported Cai ether extraction method (*9*), a chloroform extraction method, and the CORESTA recommended method (*10*). Finally, the nicotine contents in eight other leaf tobaccos of various grades, from Brazil, China, Korea, and the United States, were determined using this DSP-GC-MS method.

EXPERIMENTAL PROCEDURES

Plant Material and Reagents. Samples of tobacco were obtained from the raw materials factory of KT&G (Daejeon, Korea). These were grades B1O, B2O, and C1L, cultivated in South Korea; grades CKB4R and CKXC4O, cultivated in China; grades B3K and C4F cultivated in the United States; and grades KB4O and KC4, cultivated in Brazil.

All tobacco samples used in this test were derived from leaves from which the stems had been removed in a raw materials factory, for the manufacture of cigarettes. Leaf samples were mixed and ground to pass through a four-mesh grid using a food mixer (Hanil-FM681, Korea) to attain homogeneity.

Nicotine for use as standards was purchased from Aldrich. All organic solvents were of analytical grade and were purchased from Sigma.

Thermal Desorption Using a DSP. Nicotine fractionation was carried out using the DSP (Frontier Lab, model 2020iD), which was connected directly to the GC injector. The pyrolyzer was composed of a plunger for the sample, the sample cup, a deactivated needle (into the GC injector), and a furnace. Helium (high purity, 99.99%) was used both as the GC carrier gas and as the inert atmosphere for thermal desorption in the pyrolyzer. A microgram balance (Sartorius, model ME235S, Germany) with a shield glass to prevent external vibrations was used to precisely measure the weight of the sample (0.4 mg).

To investigate the optimum conditions, ground Korean tobacco grade B1O (0.4 mg) and internal standard *n*-heptadecane (1 μL of a 2.0 mg/mL solution in 2-propanol) were introduced into the sample cup, which was then placed in the furnace. The furnace was heated by increasing from 50 to 250, 300, or 350 °C, incrementally at 60 °C/min, at which point the nicotine component was transferred directly from the furnace to the GC-MS system.

In order to assess whether any nicotine remained in the tobacco after the desorption program, the sample was replaced in the furnace and further heating was applied from the program temperature maximum (250, 300, or 350 °C) to 400 °C, incrementally at 60 °C/min. In order to examine precision, five replicate experiments were carried out using Korean B1O grade tobacco (0.4 mg) and internal standard *n*-heptadecane (1 μL of a 2.0 mg/mL solution in 2-propanol) in the pyrolyzer sample cup, which was then placed in the furnace, and programmed from 50 to 300 °C by increasing the temperature at a rate of 60 °C/min.

Solvent Extraction Procedures. *Ether Extraction.* The procedure of Cai (*9*) was used as follows: Korean tobacco grade B1O (1.0 g) was weighed into a flask. Diethyl ether (20.0 mL) including internal standard and 5% aqueous sodium hydroxide (10.0 mL) were added, followed by internal standard *n*-heptadecane (1 μL of a 0.5 mg/mL solution in ether). After ultrasonication for 20 min, the two layers were separated and the ether layer was transferred to a vial, at which point 1 μL of the sample was injected into the GC−flame ionization detection (FID) system for analysis. Five replicate analyses were carried out using this method.

Chloroform Extraction. This extraction was performed exactly as above, except for extraction solvent and internal standard: Chloroform (20.0 mL) was the solvent, and quinoline (1 μL of a 0.5 mg/mL solution in chloroform) was the internal standard. Five replicate analyses were carried out using this method.

Hexane Extraction. The CORESTA-recommended method (*10*) was used for sample extraction using hexane as the extraction solvent. Korean tobacco grade B1O (1.00 g) was weighed into a flask. Hexane (40.0 mL), including internal standard and 6 N sodium hydroxide (10.0 mL), were added, followed by internal standard *n*-heptadecane (1 μL of a 0.5 mg/mL solution in hexane). After the sample was shaken for 1 h, the two layers were separated and the hexane layer was transferred to vial, at which point 1 μL of the sample was injected into the GC-FID system to be analyzed. Five replicate analyses were carried out using this method.

Quantitative Analysis by GC-MS. The GC-MS equipment consisted of an Agilent 6890 gas chromatograph equipped with an HP-5 MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film). The DSP was directly connected to the GC injector, which was maintained at 230 °C, with a 100:1 split ratio at the initial time. The detector consisted of an Agilent 5973 mass selective detector operating in the scan modes. Mass spectra were recorded in the electron ionization (EI) mode at 70 eV, scanning the m/z 30−500 range. Interface and source temperatures were 250 and 230 °C, respectively. The carrier gas used was helium with a controlled flow of 1.0 mL/min. The GC oven temperature was programmed from 50 (3 min) to 250 °C (20 min) by increasing the temperature at a rate of 8 °C/min. Nicotine quantitative values were obtained by using *n*-heptadecane as the internal standard.

In order to determine linearity of detector response and for quantitative analysis of nicotine, the following procedure was carried out. Four standard solutions of nicotine in 2-propanol were prepared, containing 2.5, 5.0, 7.5, and 10.0 mg/mL of internal standard and 2.0 mg/mL of *n*-heptadecane. These were introduced, in turn (1 μL), into the sample cup, which was then placed in the furnace and heated from 50 to 300 °C at 60 °C/min, at which point the components were transferred to the GC-MS instrument for analysis.

Quantitative Analysis by GC-FID. The GC-FID analysis was carried out on an HP 5890 gas chromatography equipped with split/splitless injector and using an HP-5 MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film). Temperatures of the injector and detector were 230 and 250 °C, respectively. The GC oven temperature was programmed from 50 (3 min) to 250 °C (20 min) by increasing the temperature at the rate of 5 °C/min. Samples were injected using the split mode with a split ratio of 100:1 via an autoinjector. Nicotine quantitative values were obtained by using *n*-heptadecane as the internal standard in the ether and hexane extraction methods and quinoline in the chloroform extraction method. Linearity of detector response and quantitative analysis of nicotine were carried out as in GC-MS, except that the sample (1 μL) was injected directly into the GC instrument.

RESULTS AND DISCUSSION

The nicotine content in leaf tobacco is approximately 1−3%, and nicotine accounts for about 95% of the total alkaloid fraction of a typical tobacco leaf (*16*). In recent years, various methods have been used in the analysis of nicotine and other minor alkaloids in tobacco (*5*−*10*) and many tobacco companies regard nicotine as a key component with regard to quality control. This study was performed in order to develop a DSP method that can directly analyze the nicotine content of tobacco without the use of time-consuming and labor-intensive sample preparation procedures. **Figure 1** illustrates the results of the experiments that were carried out to determine the optimum conditions for desorption of nicotine from tobacco using DSP. In these experiments, the tobacco sample and internal standard were placed in the sample cup and heated in the DSP from 50 °C to a final temperature of 250, 300, or 350 °C at a rate of 60 °C/min. It was considered that a final temperature higher than 350 °C would lead to too much thermal decomposition (*17*) and also that a final temperature below 250 °C may not desorb all of the nicotine from the sample (*17*). Additionally, a rapid heating rate was thought to be desirable, to minimize thermal decomposition and also to shorten analysis times. In order to check whether a significant amount of nicotine remained in the sample after each desorption process described above, the sample (kept at ambient temperature in the upper part of the DSP unit) was reheated from the final temperature (250, 300, or 350 °C) to 400 °C at a rate of 60 °C/min. The results are given in **Figure 1** and **Table 1**.

Inspection of **Figure 1a**,**b** and **Table 1** will show that although most of the nicotine is desorbed on heating to 250 °C,

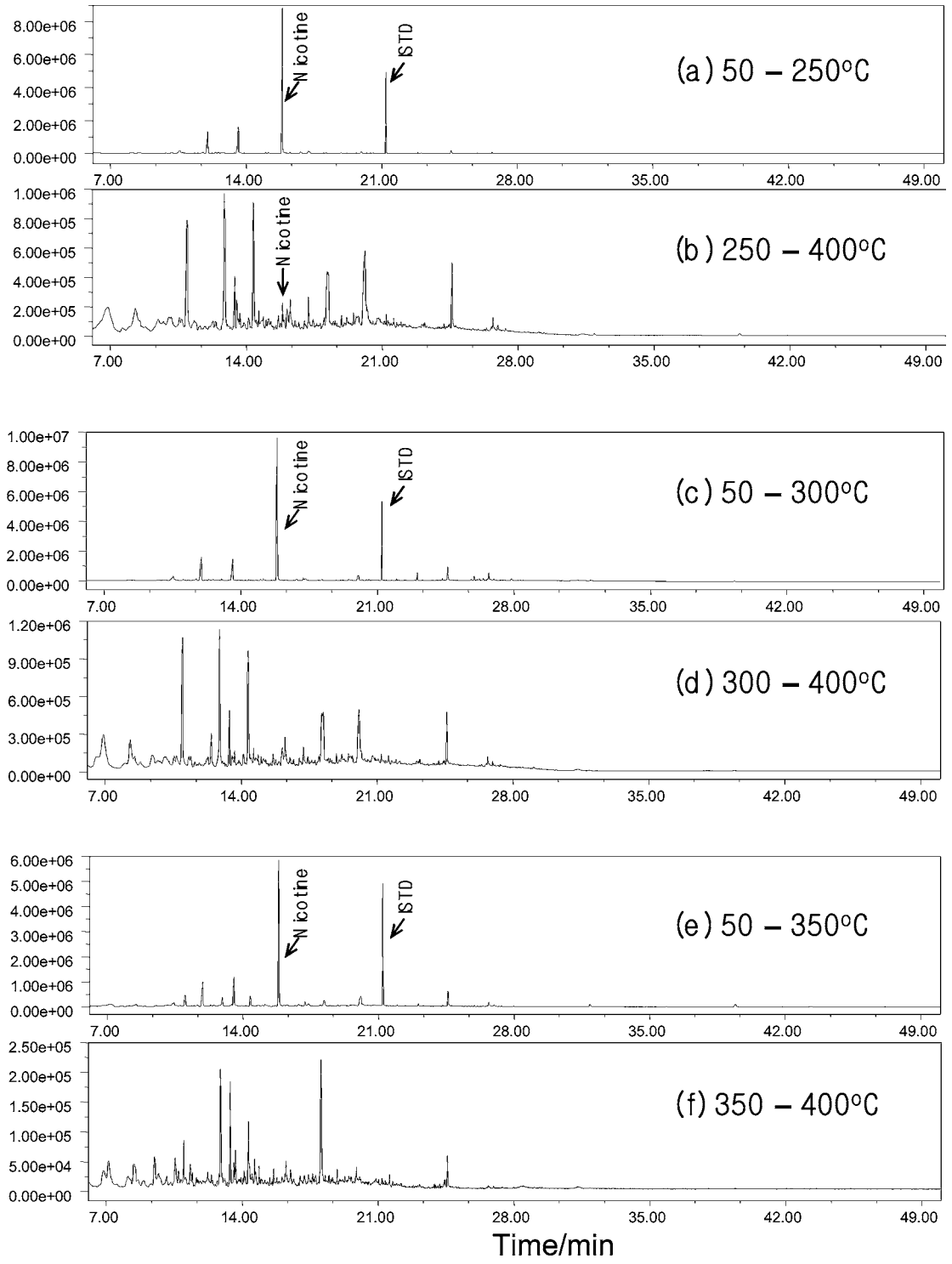

Figure 1. DSP-GC-MS TIC profile of nicotine in Korean tobacco grade B1O obtained using three DSP temperature programs. The basic programs are **a**, **c**, and **e**, and the reheating programs are **b**, **d**, and **f**. The rate of heating was 60 °C/min in all cases.

a significant amount remains undesorbed under these conditions. On the other hand, inspection of TICs **c** and **d** and **Table 1** shows that desorption of nicotine is maximized on heating to 300 °C, with no detectable amount observed in the TIC of the reheated sample (**d**). Also, TIC **c** shows only a very slight increase in the number of peaks, as compared with TIC **a**, suggesting only a little more thermal decomposition than at the lower temperature. Finally, TICs **e**, **f**, and **Table 1** indicate that heating to 350 °C actually leads to a somewhat decreased amount of desorbed nicotine and more significant amounts of thermal decomposition products are evident in TIC **e**. Thus, it

Table 1. Determination of Optimum Pyrolyzer Desorption Conditions for the Analysis of Nicotine in Korean Tobacco Grade B1O by DSP-GC-MS

DSP temperature program (°C)[a]	nicotine content (%)	DSP temperature reheating program (°C)[a]	nicotine present
50–250	1.72	250–400	detected
50–300	1.95	300–400	not detected
50–350	1.79	350–400	not detected

[a] Rate of heating, 60 °C min^{-1}.

Table 2. Replicate GC Analysis ($n = 5$) of Nicotine Content of Korean Tobacco B10, Using Optimized DSP Method and Three Solvent Extraction Methods

method	DSP[a]	ether[b]	chloroform[c]	hexane[d]
	1.95	1.87	1.96	1.66
	1.92	1.89	1.99	1.71
(nicotine %)	2.00	1.83	1.95	1.62
	1.93	1.95	1.98	1.62
	1.98	1.82	1.99	1.63
mean	1.96	1.87	1.98	1.65
RSD %	1.9	2.7	0.91	2.3

[a] Double-shot pyrolysis-GC-MS. [b] Ether extraction-GC-FID. [c] Chloroform extraction-GC-FID. [d] Hexane extraction-GC-FID (CORESTA method).

would appear that the optimum DSP conditions for the analysis of nicotine in tobacco using this method are heating from 50 to 300 °C, at a rate of 60 °C/min.

The calibration curve for the standard nicotine solutions (2.5−10.0 mg/mL) was linear with a correlation coefficient (R^2) of 0.999, when the analysis was carried out under the optimum conditions, as described above. This coefficient value is very high as compared to that of 0.983 obtained by Rosa, using the fixed temperature Victoreen pyrolyzer (*11*). The main reasons for the better linearity and accuracy of our method are probably due to our use of an external standard and our use of a temperature program, rather than a fixed temperature for nicotine desorption.

In order to test the precision of the optimized method, five replicate analyses were carried out on Korean tobacco, grade B1O. The results are displayed in **Table 2**, where it can be seen that the average value for the five determinations was 1.96% (1.96 g per 100 g of tobacco) with a relative standard deviation (RSD) of 1.9%. Cai ether extraction (*9*), hexane extraction (CORESTA) (*10*), and chloroform extraction methods were applied to the analysis of the same grade of Korean tobacco in order to verify the reliability of the DSP method. Five replicate analyses were carried out for each method, as described in experimental procedures: The results are displayed in **Table 2**, and typical chromatograms are shown in **Figure 2**. The ether extraction method is a currently accepted procedure in tobacco analysis laboratories, whereas the hexane extraction method is a recommended method of CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco, an association founded in France in 1956 for the promotion of international cooperation in tobacco research). The chloroform extraction method used in this study was a slightly modified version of the Cai ether extraction method (*9*): Quinoline was used as the internal standard, rather than *n*-heptadecane. In all of these solvent extraction methods, it was necessary to increase the pH value of the sample solutions in order to extract nicotine efficiently from tobacco. Aqueous sodium hydroxide was used in all three methods for this purpose. In **Table 2**, it can be seen that the mean nicotine content of grade B1O Korean tobacco (1.96%) determined by the DSP method is in close agreement with that of the chloroform extraction method (1.98%), whereas both the ether extraction and the hexane extraction methods give rather lower mean values (1.87 and 1.65%, respectively), although the agreement can still be considered reasonable and fair, respectively. The differences in the solvent extraction results can be explained partially by the different relative densities of the solvents: The low mean extraction of nicotine from tobacco by diethyl ether and hexane may be, in part, due to loss by evaporation, as these two solvents are less dense than aqueous sodium hydroxide and form the upper layer. Also, it is possible that hexane, being less polar than the other two solvents, is slightly less efficient at extracting nicotine from tobacco. Chloroform is denser than aqueous sodium hydroxide and so forms the lower layer, thereby offering less opportunity for loss by evaporation.

From **Table 2**, it is apparent that all four methods possess good precision, with the chloroform extraction method being the best (RSD = 0.91%). However, the DSP method, with RSD of 1.9%, compares favorably with the ether extraction and hexane extraction methods (RSD = 2.7 and 2.3%, respectively). The lower precision of the latter two methods may be explained by the extra uncertainty caused by evaporation losses from the upper layers, especially in the case of ether, which is more volatile than hexane.

Davoli et al. reported a precision (RSD) ranging from 1.64 to 13.30% when nicotine was analyzed using an SPME-GC method (*18*). Also, Lee et al. demonstrated that precision (RSD) ranged from 2.4 to 7.8% when nicotine was analyzed using GC-MS, after extraction of tobacco with methanol:chloroform (1:3, v/v) (*16*). Finally, Yang et al. reported a precision (RSD) of 2.4% when nicotine was analyzed using a GC-NPD method (*8*). On the basis of these results, the precision (1.9%) of the DSP-GC-MS method used in this study is considered to be excellent. Because the DSP-GC-MS method uses a small sample (0.4 mg), it is necessary to guarantee the homogeneity of the sample. It should be possible to perform precise analyses of nicotine content for manufactured cigarettes, chewing tobaccos, and other tobacco products, provided the sample is fully mixed and uniformed by milling it as powder. Additionally, volatile tobacco components other than nicotine can be analyzed by the DSP-GC-MS method and it is possible that such information could be used in formulation or analysis of blends.

In order to investigate the nicotine contents of different types of flue-cured tobacco, various products from different countries were analyzed using the DSP-GC-MS method. These were KC4F and KB4O produced in Brazil, CKXC4O and CKB4R produced in China, C2L and B2O produced in Korea, and C4F and B3K produced in the United States. The results are shown in **Table 3**, where it can be seen that tobacco leaf grade CKB4R (China) and KB4O (Brazil) have the highest nicotine contents (2.19 and 2.13%, respectively), followed closely by B3K (United States) and B2O (Korea) with 2.05 and 2.03%, respectively. In tobacco leaf grade nomenclature, the letter C indicates leaves taken from the lower stem and B is used for leaves taken from the upper stem. It can be seen from **Table 3** that the lower stem leaf grades consistently have lower nicotine contents. Jang et al. have previously reported nicotine contents of 1.20−1.36% for flue-cured Korean leaves of grade C2L, using a solvent extraction-GC method (*15*). These results are in good agreement with our results (1.21%) using the DSP-GC-MS method.

In conclusion, the optimal DSP desorption conditions for the analysis of nicotine in tobacco using a DSP-GC-MS method required a heating program of 50−300 °C, at a rate of 60 °C/min. Replicate analyses ($n = 5$) of Korea tobacco grade B1O resulted in a mean nicotine content of 1.96% with a RSD of 1.9%. Both of these values compare favorably with the results of three solvent extraction-GC-FID methods: 1.98% (0.91% RSD) from the chloroform extraction method, 1.87% (2.7% RSD) from the ether extraction method, and 1.65% (2.3% RSD) from the hexane extraction (CORESTA-recommended) method. Furthermore, the DSP-GC-MS method has some advantages over the solvent extraction methods: It is much more rapid (by a factor of about 2), it is less labor-intensive, and it requires very small samples (less than 0.5 mg). In addition, this method

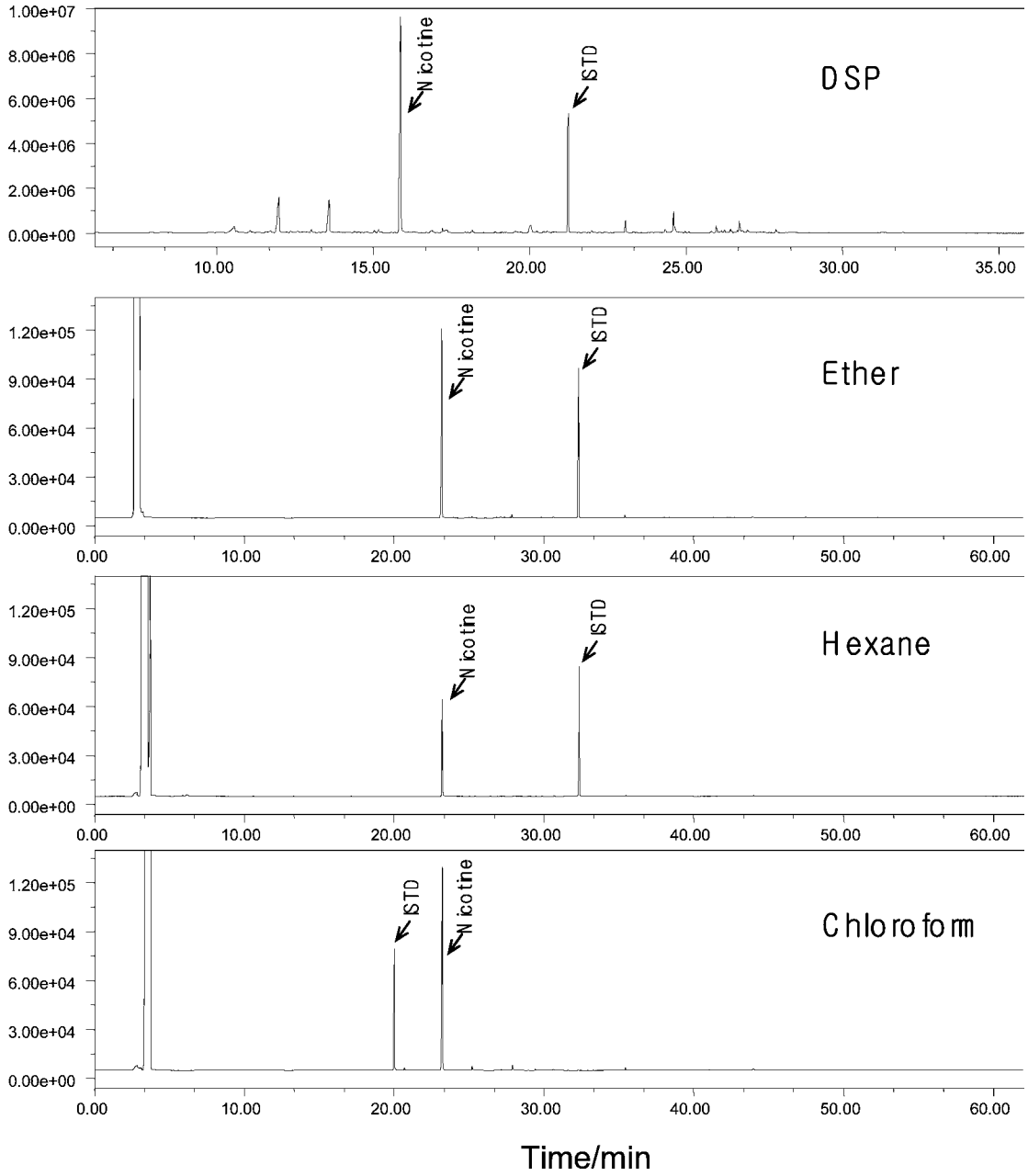

Figure 2. Chromatograms of nicotine in Korean tobacco grade B1O, determined by the DSP-GC-MS method and solvent extraction-GC-FID methods.

Table 3. Nicotine Content of Tobaccos from Four Different Countries Using DSP-GC-MS

country of origin	grade	nicotine (%)[a]
Korea	C2L	1.21
	B2O	2.03
USA	C4F	1.46
	B3K	2.05
Brazil	KC4F	1.73
	KB4O	2.13
China	CKXC4O	1.98
	CKB4R	2.19

[a] RSD = 1.9%.

does not require the use of solvents to analyze the nicotine content of tobacco (except for a small amount of 2-propanol in which the internal standard is dissolved) and hence is less hazardous and "greener" than the solvent extraction methods. Therefore, we propose that this method will be better suited to quality control than the solvent extraction methods. The fact that very small samples can be analyzed accurately may render this method suitable for forensic analysis of tobacco samples found at the scene of a crime.

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