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NICOTINE DETERMINATION FROM TABACCO BY GC/MS

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Abstract

The paper presents the results of the quantitative determination of nicotine in 39 tobacco samples and tobacco products through the practical application of a validated GC/MS method. The isolation of nicotine was performed by extraction with methanol/dichloromethane 1/1 (v/v) and it was analysed by GC/MS using a DB 5 MS column (30 m x 0.25 mm, 0.25 μ m), carrier gas helium; the detection was performed by mass spectrometry, MSD's (mass spectrometric detector) source temperature was 230°C and the MSD's quadrupole temperature was fixed at 150°C, in SCAN mode. The method's validation was performed through the parameters: linearity, accuracy, precision, limits of detection and quantification. The values of nicotine content determined in cigarettes (31 samples) ranged between 10.14 and 21.15 mg nicotine/g of tobacco, with the average 15.35; the concentrations in cigars and pipe tobacco (6 samples) ranged between 13.15–28.38 mg nicotine/g of tobacco (average 22); in tobacco leaves, the registered average content of nicotine was 26.79 mg /g of tobacco.

Rezumat

Lucrarea prezintă rezultatele analizei cantitative ale nicotinei din 39 probe de tutun și produse de tutun prin aplicarea în practică a unei metode validate GC/MS. Izolarea nicotinei s-a realizat prin extracție cu metanol/diclorometan 1/1 (v/v); probele au fost analizate prin GC/MS, utilizând o coloană DB 5 MS (30 m x 0,25 mm; 0,25 μ m), faza mobilă heliu. Detecția s-a realizat prin spectrometrie de masă cu temperatura sursei MSD (mass spectrometric detector) de 230°C, cea a cvadropolului MSD de 150°C, în modul SCAN. Validarea metodei s-a făcut prin parametrii: liniaritate, exactitate, precizie, limită de detecție și cuantificare. Concentrațiile nicotinei determinată din țigărete (31 probe) au variat între 10,14 și 21,15 mg nicotină/g de tutun, cu o medie de 15,35; concentrațiile în țigările de foi și tutun de pipă (6 probe) au variat între 13,15–28,38 mg nicotină/g de tutun, cu o medie de 22 mg/g; în frunzele de tutun s-a înregistrat un conținut mediu de nicotină de 26,79 mg/g de tutun.

Keywords: nicotine, tobacco, GC/MS

Introduction

Nicotine is the major tobacco alkaloid; it is found in proportion of about 1.5% of the tobacco weight from commercial cigarettes, and represents approximately 95% of the total tobacco alkaloids [4]. Nicotine represents the major cause of intoxication; it is considered that during smoking one cigarette, 3-4 mg of nicotine pass into the airways, of which 90% are reabsorbed into the lungs [3, 10]. Smokers are able to vary the intake of smoke to satisfy their needs by adjusting the parameters: the brands of cigarettes selected, number of cigarettes smoked, and number of puffs per cigarette, depth and duration of inhalation. Smoking affects seriously, most often irreversibly, the respiratory and cardiovascular system, the eyes, the central nervous system, the digestive system and the skin. It is considered that the life expectancy of a smoker compared to a non-smoker's is eight years shorter [5, 11].

Nicotine causes psychic addiction, which is installed faster than alcohol and other drugs. Addictive properties of nicotine can be explained by activation of the reward circuit, nicotine increases the dopamine level in this circuit, especially in the *nucleus accumbens*, substrate for pleasant sensations felt by smokers. Maintaining high levels of dopamine causes the desire to repeat the consumption [2]. Tobacco consumption remains the leading cause of preventable death, globally contributing to approximately 4 million deaths each year [6]. In Romania, a GATS (Global Audit Tobacco Survey) study conducted by the Ministry of Health has established a significant prevalence of tobacco use, approximately 5 million smokers, out of which 4.5 million daily smokers.

This paper presents the quantitative analysis of nicotine from cigarettes, cigars, pipe tobacco and tobacco leaves using a GC/MS method validated previously [17].

Materials and Methods

Materials:

A number of 39 samples of tobacco were analysed, namely:

- 31 samples of different types of cigarettes, corresponding to 15 brands, purchased from local retail points (Table I);
- five samples of cigars (Table II, samples 1-5);
- one sample of pipe tobacco (Table II, samples 6);
- two samples of tobacco leaves, from two geographical regions (Timisoara and Iasi, Romania) (Table III).

Reagents: methanol HPLC (Sigma), dichloromethane HPLC grade (Promochem), nicotine for synthesis (Merck).

Equipment:

- IKA Werke Vibrax VXR vortex;
- Agilent Technologies 7890 A Gas Chromatograph equipped with Agilent Technologies 5975C inert MSD detector;
- DB 5 MS column (30 m x 0.25 mm I.D., 0.25 µm film thickness);
- Software: Chemstation and Wiley mass spectra library.

Chromatographic conditions:

- Temperature program: the initial temperature was 100°C, which increased with 10°C/min to 190°C, and then with 20°C/min to 280°C, then it was constant for 5 min;
- Injection port temperature: 250°C;
- Split ratio: 1/10;
- Transfer line temperature: 280°C;
- Carrier gas: helium, at a flow rate of 1 mL/min;
- MSD source temperature: 230°C;
- MSD quadrupole temperature: 150°C;
- Detection in SCAN mode.

Sample preparation

The samples of tobacco (1.0-1.5 g) were extracted in a mixture of methanol/dichloromethane (1/1, v/v) by stirring for one hour at 500 rotations/min. After filtration, the solution was diluted at 50 mL, followed by another dilution of 1 mL to 4 mL with the same solvent; 0.3 µL of these solutions were injected in the GC/MS instrument in the mentioned conditions. Three measurements were made for each sample. The nicotine content was expressed in mg/g of tobacco. The statistical evaluation of the results was performed using Microsoft Excel.

Results and Discussion

The GC/MS method was validated according to the data from literature [8, 14, 15]; the results were as follows: the method is linear over the range 1.01 to 201.8 µg/mL; the detection limit is 3.6 µg/mL; the quantification limit is 10.8 µg/mL; the method is precise (RSD = 0.6562%, n=9) and accurate (mean recovery = 100.28% between 99.08 to 100.96%) [17]. In the study of linearity, the calibration curve equation obtained is:

$$\text{Peak area} = 251535.6 \times \text{concentration } (\mu\text{g/mL}) + 8102784$$

or

$$\text{Concentration } (\mu\text{g/mL}) = \frac{\text{Peak area} - 8102784}{251535.6}$$

In the second part of this study, the method was applied to determine the nicotine from tobacco extracts. After the GC/MS analysis, the peak of nicotine was identified and the peak's area was measured. In figure 1 it is presented, the chromatogram for the "Jewels[®] vanilla" cigar sample.

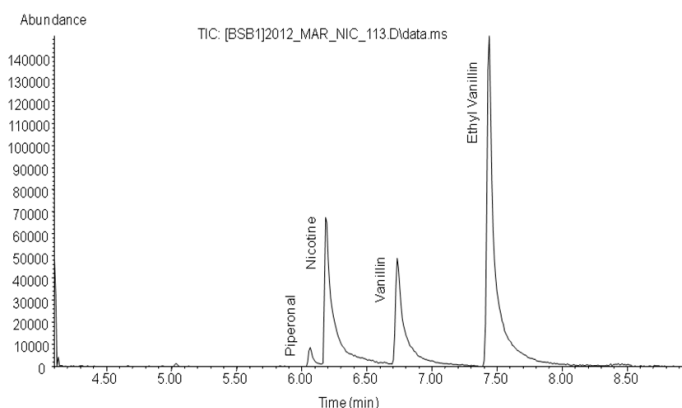


Figure 1

Gas chromatogram for "Jewels[®] vanilla" cigar sample

In the conditions of the proposed method (extraction in mixture of methanol/dichloromethane (1/1, v/v) and analysis by GC - MS, there are no additional peaks at retention times close to that of nicotine (approximately 6.2 minutes). Furthermore, by comparing the mass spectrum of the peak corresponding to nicotine with those from spectral libraries (Wiley) a high purity was obtained (over 98%). These arguments demonstrate that other components present in samples of tobacco (alkaloids or other compounds) do not interfere.

Using the equation of linearity, the concentration in solution (μg nicotine/mL) and the corresponding concentrations in tobacco (mg nicotine/g of tobacco) were calculated. The nicotine content from tobacco was evaluated using the formula:

$$\text{mg nicotine/g tobacco} = \frac{N \times 4 \times 50}{T \times 1000}$$

In this formula "N" represents the nicotine concentration ($\mu\text{g/mL}$) calculated from the equation of the calibration curve and "T" represents the amount of analysed tobacco. The results of the measurements obtained are shown in Tables I, II and III.

Table I
Nicotine content in cigarette samples

Nr. crt.	Sample name	Nicotine content printed on the pack mg/cigarette	Tobacco quantity (g)"T"	Average peak area (n=3)	Nicotine in solution (µg/mL) "N"	Nicotine in tobacco (mg/g)	RSD % (n=3)
1	Camel® blue	0.5	1.1278	33380264	100.49	17.82	0.0990
2	Camel® red	0.8	1.1831	39572781	125.11	21.15	0.0221
3	Chesterfield® blue	0.7	1.0478	23211054	60.06	11.46	0.3064
4	Chesterfield® red	0.8	1.1792	28177695	79.81	13.54	0.1339
5	Davidoff® red	1	1.2365	39145859	123.41	19.96	0.1262
6	Dunhil® white	0.5	1.2595	29182195	83.8	13.31	0.1152
7	Dunhil® blue	0.6	1.2737	30780809	90.16	14.16	0.0897
8	Dunhil® black	0.9	1.2858	37492815	116.84	18.17	0.1762
9	Dunhil® red	1	1.2521	39941636	126.58	20.22	0.1211
10	Kent® silver	0.1	1.1318	22542832	57.41	10.14	0.0696
11	Kent® white	0.4	1.2408	25423412	68.86	11.1	0.0878
12	Kent® blue	0.7	1.205	32470311	96.88	16.08	0.1137
13	Kent® nanotek	0.6	1.1838	23768270	62.28	10.52	0.1980
14	LM® blu 83	0.6	1.1824	25897466	70.74	11.97	0.1971
15	LM® red label	0.8	1.185	35674048	109.61	18.5	0.0872
16	Lucky strike® red	0.8	1.2786	39250601	123.83	19.37	0.0490
17	Marlboro® gold advance	0.7	1.2712	32102094	95.41	15.01	0.0828
18	Marlboro® gold original	0.5	1.2886	28444656	80.87	12.55	0.1203
19	Marlboro® red	0.8	1.2783	40504130	128.81	20.15	0.0544
20	Monte Carlo® green	0.7	1.1576	28317779	80.37	13.88	0.1146
21	Monte Carlo® red	0.8	1.2753	36618572	113.37	17.78	0.0990
22	Pall Mall® blue	0.6	1.1128	30138780	87.61	15.75	0.1013
23	Pall Mall® orange tek charcoal filter	0.6	1.1315	28608218	81.52	14.41	0.0154
24	Pall Mall® superslim blue	0.3	1.4876	31518834	93.09	12.52	0.1967
25	Parliament®	0.6	1.2228	32669425	97.67	15.97	0.0580
26	Philip Morris® white quantum silver	0.8	1.0797	33528166	101.08	18.72	0.1077
27	Philip Morris blue quantum blue	0.7	1.1411	34060057	103.2	18.09	0.1164
28	Vogue® red balade au parc	0.4	1.4296	27774492	78.21	10.94	0.0757
29	Winchester® red	0.8	1.253	33372986	100.46	16.04	0.0770
30	Winston® blue	0.5	1.0453	27644052	77.69	14.86	0.0537
31	Winston® light	0.6	1.01	22852538	58.64	11.61	0.1854

Table I contains the trade name of the 31 samples of cigarettes, the nicotine content printed on the pack (mg/cigarette), the amount of tobacco used for analysis ("T") (g), the average of the peak area for a number of three determinations (n) for each sample, the concentrations of nicotine in tobacco extracts ("N") ($\mu\text{g/mL}$), the nicotine concentrations that were determined in cigarettes (mg nicotine/g of tobacco) and RSD% values.

The nicotine content printed on the package (mg/ cigarette), represents the nicotine content determined by "smoking machine" from the cigarette smoke in standard conditions: one puff per minute, with a duration of 2 seconds, the inhaled volume of 35 mL, to compare the toxicity of different types of cigarettes [7, 18].

The results presented in Table I show that Kent[®] silver cigarette had the lowest nicotine concentration 10.14 mg/g while the maximum concentration was found in Camel[®] red 21.15 mg/g, respectively. The average concentration of nicotine in the analysed brands was 15.35 mg/g. The obtained results correspond to those from the literature [9, 12, 13, 16, 19, 20]. Kozłowski et al. have shown that the content of nicotine in cigarettes is 10.2 mg/g for the U.S. brands, 13.5 mg/g for Canadian brands and 12.5 mg/g for British brands [12]. Fukumoto et al. in their study on Japanese filter cigarettes, found 11.72 ± 2.27 mg nicotine/cigarette [9]. Using a LC-MS-MS method, Vlase L. et al. have analysed 40 types of cigarettes. The nicotine content ranged from 7.63 to 17.67 mg nicotine / g of tobacco [19].

The variation of nicotine content among different commercial brands of cigarettes is attributed to the different varieties of tobacco used for the manufacturing; also the filling composition (type of tobacco, use of expanded tobacco, reconstituted tobacco) can vary greatly from brand to brand [18].

The nicotine content of cigarettes may be influenced by temperature and humidity conditions during transport and storage.

Table II presents the nicotine content in cigars (samples 1-5) and pipe tobacco (sample 6). The nicotine concentrations ranged from 28.38 mg/g in "Amphora[®] pipe tobacco" to 13.15 mg/g in "Scandinavian[®] café crème" cigars.

Table II
Nicotine content in cigars and pipe tobacco

Nr. crt.	Sample name	Tobacco quantity (g) "T"	Average peak area (n=3)	Nicotine in solution (µg/mL)"N"	Nicotine in tobacco (mg/g)	RSD % (n=3)
1	Black stone [®] winer	1.3017	49706767	165.4	25.41	0.0519
2	Café crème [®] scandinavian	1.2065	28050496	79.3	13.15	0.1279
3	Jewels [®] vanilla	1.2924	49472443	164.47	25.45	0.0792
4	King Edward [®] (cigars of leaf)	1.153	44326846	144.01	24.98	0.1306
5	Villiger [®] premium no 4 Sumatra	1.268	31447490	92.81	14.64	0.0734
6	Amphora [®] pipe tobacco	1.2023	51020341	170.62	28.38	0.1124

At the same time, nicotine was also determined in two samples of Burley[®] tobacco leaves from two geographical regions from Romania (Timisoara and Iasi) (Table III).

Table III
Nicotine content in tobacco leaves

Nr. crt.	Name of variety / region of origin	Tobacco quantity (g) "T"	Average peak area (n=3)	Nicotine in solution (µg/mL)"N"	Nicotine in tobacco (mg/g)	RSD % (n=3)
1	Burley [®] tobacco/ Timișoara	1.2181	48872454	162.08	26.61	0.0230
2	Burley [®] tobacco/ Iași	1.6475	63973651	222.12	26.96	0.0832

The data presented in Table III show close values of nicotine content to the samples of tobacco leaves. The nicotine concentration in tobacco leaves from Timisoara was 26.61 mg/g (RSD = 0.0230%, n = 3) and in tobacco leaves from Iasi was 26.96 mg/g (RSD = 0.0832%, n = 3). The obtained results correspond to those from the literature [1].

In Table IV there are tabulated the minimum, average and maximum measurements of nicotine content in tobacco products (cigarettes, cigars, pipe tobacco), and tobacco leaves.

Table IV

The minimum, average and maximum measurements of nicotine content from cigarettes, cigars, pipe tobacco and tobacco leaves

mg nicotine / g tobacco	Cigarettes	Cigars and pipe tobacco	Tobacco leaves
Min	10.14	13.15	-
Average	15.35	22	26.79
Max	21.15	28.38	-

The highest average of nicotine content 26.79 mg/g was found in tobacco leaves, and the lowest average 15.35 mg/g was found in cigarettes.

Conclusions

The content in nicotine from 39 tobacco samples was determined by using a GC/MS method, validated by the authors.

For the 31 tobacco samples corresponding to 15 cigarette brands, the variation limits of the nicotine content were more restricted (10.14 - 21.15 mg/g) in comparison with the 6 samples of cigars and pipe tobacco for which the nicotine content varied in a larger range of values, 13.15 - 28.38 mg/g. For the two samples of tobacco leaves, the nicotine's concentration registered very close values.

According to the average values, the highest nicotine content was registered in the case of the tobacco leaves (26.79 mg/g), followed by cigars and pipe tobacco (22 mg/g), and by the cigarettes (15.35 mg/g).

The obtained results are in accordance with literature and confirm the method's applicability, which will be extended by the authors also to determine nicotine in biological samples.

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