STUDIA UNIVERSITATIS BABEŞ-BOLYAI, PHYSICA, L, 4b, 2005

# DETERMINATION OF NICOTINE FROM TOBACCO BY LC-MS-MS

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**ABSTRACT**

A new high performance liquid chromatography coupled with mass spectrometry method (LC/MS/MS) for quantification of nicotine from tobacco was elaborated. It was utilized an Atlantis HILIC, 100 mm x 3.0 mm i.d., 3 μm column with a mobile phase containing acetonitrile/ solution 0.2% formic acid in water. The ionization was optimized using ESI(+) and enhanced selectivity was achieved using tandem mass spectrometric analysis. The precursor to product ion transitions of m/z 163 -> (105.8, 131.8) were used to measure the nicotine concentrations. The quantification was made using the external standard method. The calibration curves were made on range 0.04-4 μg/ml. Nicotine content was determined in 40 brands of cigarettes available in Romania. With our methodology, we obtained values of nicotine in tobacco between 7.5 and 17.6 mg/g.

## INTRODUCTION

Tobacco has been smoked for at least the last three thousand years. Christopher Columbus found it when he landed in the Americas in 1492, but ancient temple carvings show tobacco being smoked in Central America as long ago as 1,000 BC.

Nicotine, (S)-3-(1-methyl-2-pyrrolidinyl)pyridine, is the most abundant of the volatile alkaloids in the tobacco leaf. The primary commercial source of nicotine is by extraction from the plant Nicotinia tabacum and Nicotinia rustica. Nicotine acts on nicotinic cholinergic receptors, affects most organ systems in the body and is a highly addictive drug [1]. Nicotine normally makes up about 5 percent of a tobacco plant, by weight. Cigarettes contain 8 to 20 milligrams (mg) of nicotine (depending on the brand), but only approximately 1 mg is actually absorbed in the human body.

Approximately 70% of smokers who want to quit smoking cannot and about 83% of smokers smoke every day [2]. A study done by the Center for Health Policies and Services in Romania showed that 39.9 percent of Romanians smoke daily.

The amount of information regarding nicotine content in cigarettes is slight and only a few studies have been done to bring light to this subject [3]. There were described HPLC methods for the determination of nicotine from pharmaceutical formulations [4] and HPLC-tandem mass spectrometry methods for determination from biological samples [5,6].

The aim of our study was to elaborate a rapid and simple LC/MS/MS method suitable for quantification of nicotine in a large number of tobacco samples.

## MATERIALS AND METHOD

### Chemicals

Nicotine was purchased from Sigma-Aldrich (Germany). Acetonitrile and formic acid 98% were from Merck KGaA (Darmstadt, Germany).

### Apparatus and chromatographic conditions

An Agilent 1100 Series (Agilent, USA) chromatographic system was used (binary pump, online degasser, autosampler, thermostat set at 35 °C and 1100 LC/MSD Ion Trap detector). Analytical column: Altantis HILIC, 100 mm x 3.0 mm i.d., 3.0 µm, (Waters, USA). The mobile phase was a mixture 80:20 (v/v) of acetonitrile: 0.2% formic acid in water. The flow rate was 1 ml/min and the injection volume of 5 µl. The mass analyser operated with an ESI source, ion mode: positive, Vcap: 7000V, nebulizer: 70 psi, drying flow gas: 12 l/min, drying gas temperature: 325 °C, max accumulation time: 100 ms, ion target: 25000, scan range: 70-170 m/z. The precursor to product ion transitions of m/z 163 -> (105.8, 131.8) were used for nicotine quantification.

### Standard solutions

Stock solution of nicotine (20 mg/ml) was obtained in acetonitrile. Seven standard solutions (0.04-4 µg/ml) were obtained by diluting appropriate volumes of stock solution with acetonitrile / 0.2 % formic acid 80/20 (v/v).

### Sample preparation

About 50 mg tobacco were accurately weighted and introduced in a 15 ml centrifuge tube. 2 ml of $H_2SO_4$ 0.1M solution were added and the mixture kept in ultrasonic bath for 10 seconds. Then, the centrifuge tube was introduced in a water bath at 70 °C for 10 minutes. In a 2 ml Eppendorf tube, 50 µl of extract were diluted to 1 ml with acetonitrile. The tube was centrifuged for 6 min at 5000 rpm and 50 µl of supernatant was diluted to 1 ml with mobile phase. 5 µl from final solution were injected in chromatographic system.

## RESULTS AND DISCUSSIONS

It is well known that ESI-LC/MS/MS detection is a very selective and sensitive analytical technique for quantification of easily ionisable organic compounds. In EI-MS technique, the compound is ionized after impact with high-

energy electrons and the $M^+$ ion is first obtained and immediately fragmented into daughter ions. Almost any organic structure can be analyzed in this way. However, in case of ESI-MS analysis, the processes are very different. First, only compounds that are ionisable in solution can be detected. The ionisation occurs easily when the compound of interest has either basic or acidic functions and the pH of media is adjusted properly. Second, the ions formed in solutions are always adducts like $(M+H)^+$ or $(M-H)^-$. These ions can be isolated by mass spectrometer and then fragmented by "shaking" them at high vacuum, in presence of argon, using a voltage specific for m/z value of the ion. Multiple steps of isolation-fragmentation can be done, that allow obtaining so-called MSn spectra (n=2..5 ), which are often more informative than classical EI-MS spectra.

The MS2 spectrum of nicotine obtained after isolation and fragmentation of m/z 162.9 is shown in Fig. 1. The most abundant ions, 105.8 and 131.8 were chosen for quantification.

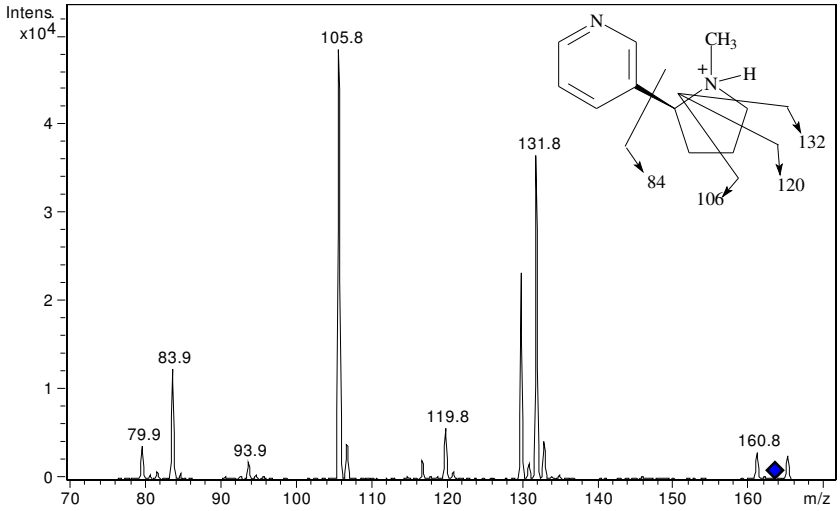
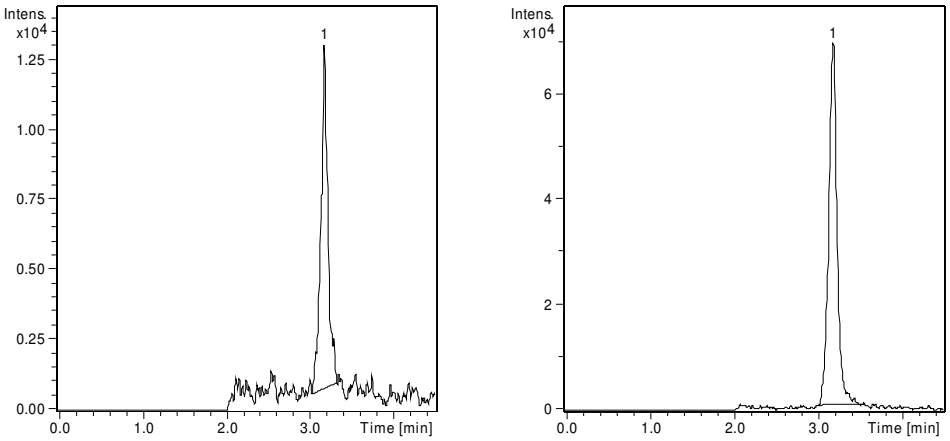



Fig. 1. MS2 spectrum of nicotine and the proposed fragmentation paths

A typical chromatogram for a standard solution of nicotine at quantification limit (concentration 0.04 µg/ml, 100 pg injected on column) and for an extract from tobacco leafs is shown in Fig. 2.




(a) (b)

Fig. 2. (a) Typical chromatogram of nicotine at quantification limit (0.04µg/ml, 100 pg on column); (b) Chromatogram of nicotine extracted from tobacco leafs; RT= 3.1 min

The mobile phase composition was optimized in order to obtain a good peak shape and a short analysis time. At a composition of acetonitrile/ formic acid 0.2% 80/20 (v/v), the retention time for nicotine was 3.1 min and these conditions were selected for calibration and quantification.

**Table 1.**

Nicotine content (mg nicotine/ g tobacco) in 40 brands of cigarettes

Using this analytical method, 40 samples of tobacco from commercial products were analyzed. From each brand, two replicates were made. The nicotine content in commercial tobacco (ascending order), expressed as mg nicotine / g tobacco, is presented in Table 1.

| No | Name | Producer | mg nicot/g tobacco |
|---|---|---|---|
| 1 | Carpati Green | Galaxy Tobacco SA | 7.63 |
| 2 | Slims Prestige | Bulgartabac | 8.68 |
| 3 | Snagov | Galaxy Tobacco SA | 8.74 |
| 4 | Next Ligths | Philip Morris | 8.83 |
| 5 | L&M Rosu | Philip Morris | 9.16 |
| 6 | L&M Lights Menthol | Philip Morris | 9.61 |
| 7 | Red & White | Philip Morris | 9.71 |
| 8 | Kossuth | SC Continental Tobacco Romania SA | 9.82 |
| 9 | Marlboro | Philip Morris | 9.86 |
| 10 | Vlah | N/A | 10.03 |
| 11 | Assos | Philip Morris | 10.10 |
| 12 | Saint George | Gallaher Romania SRL | 10.13 |
| 13 | Pannonia albastru | SC Continental Tobacco Romania SA | 10.41 |
| 14 | Pannonia rosu | SC Continental Tobacco Romania SA | 10.48 |
| 15 | L&M Lights | Philip Morris | 10.53 |
| 16 | Parliament Lights | Philip Morris | 11.07 |
| 17 | Kent Blue 8 | British American Tobaco | 11.12 |
| 18 | Pall Mall Ligths | British American Tobaco | 11.33 |
| 19 | Pall Mall Ultra Lights | British American Tobaco | 11.57 |
| 20 | Viceroy Rich | British American Tobaco | 11.64 |
| 21 | Kent Mintek 2 | British American Tobaco | 12.35 |
| 22 | Rothmans Royals | British American Tobaco | 12.49 |
| 23 | Lucky Strike | British American Tobaco | 12.60 |
| 24 | Dunhill | British American Tobaco | 13.21 |
| 25 | Kent Gold 1 | British American Tobaco | 13.28 |
| 26 | King Edward | Swisher International Inc. | 13.39 |
| 27 | Camel | JT International Manufacturing | 13.44 |
| 28 | Viceroy Special | British American Tobaco | 13.45 |
| 29 | Davidoff | Davidoff&Cie SA | 13.55 |
| 30 | Winston Lights | JT International Manufacturing | 13.55 |
| 31 | Parliament Extra Lights | Philip Morris | 13.62 |
| 32 | Pall Mall Menthol | British American Tobaco | 13.66 |
| 33 | Winston | JT International Manufacturing | 13.72 |
| 34 | West Ice | Reemtsma | 13.99 |
| 35 | Marlboro Ligths Menthol | Philip Morris | 14.06 |
| 36 | Rothmans King Size | Pall Mall | 14.13 |
| 37 | Marlboro Ligths | Philip Morris | 14.51 |
| 38 | Muratti Ambassador | Philip Morris | 14.57 |
| 39 | Winchester | JT International Manufacturing | 15.64 |
| 40 | Modern | Modern Cigarettes Inc | 17.67 |

A global problem on the international market is the trade with counterfeit cigarettes. These contain more nicotine than normal, even with 28% higher content and 75% tar content, which may cause serious health problems. On the other hand, the tobacco used can be of poor quality and the content in nicotine smaller. The analyses made, showed in one product a quantity of nicotine smaller in the normal cigarettes than in the light type of the same brand. The Romanian cigarettes have proved to possess a weak concentration of nicotine.

## CONCLUSION

The aim of this study was to elaborate a rapid and simple LC/MS/MS method for quantification of nicotine in tobacco.

Chromatographic, detection and extraction parameters were optimized in order to obtain high selectivity and a short run-time. The procedure of sample processing method was also simplified, allowing preparation of a great number of samples in a short time.

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