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A SIMPLE TLC METHOD FOR EVALUATION OF NICOTINE IN CIGARETTES

ANAMARIA HOSU[a], CLAUDIA CIMPOIU[a, *]

ABSTRACT. In this study, a rapid and sensitive thin-layer chromatographic method for evaluation of nicotine content in twelve popular cigarettes from Romanian market has been used. The TLC separation was performed on silica gel F254 plates using a mixture of ethyl acetate - methanol - conc. ammonia - water 80:25:0.2:15.8, v/v/v/v as mobile phase. Detection was made in UV light at 254nm and in visible light after derivatization with Dragendorff's reagent and the areas of the characteristic bands of nicotine were determined using ImageJ software. The results showed a significant variation of nicotine content in investigated cigarettes, but in good agreement with the concentrations of nicotine specified on the label.

Keywords: *nicotine evaluation, thin-layer chromatography, cigarettes*

INTRODUCTION

The highly toxic compound from tobacco alkaloids is nicotine, 3-(1-methyl-2-pyrrolidinyl) pyridine, a colorless, less to pale yellow, hygroscopic oily liquid present in the leaves of *Nicotiana tabacum*. Christopher Columbus have been found tobacco in the Americas in 1492, but historical sites show that tobacco has been smoked in Central America for at least three thousand years. Nicotine is a highly stimulant of central and peripheral nervous system generating short-term adverse health effects, like elevated blood pressure, heart rate, and blood glucose [1]. Long-term tobacco use is associated with increased cancer rates, incidence of atherosclerotic arterial disease, chronic obstructive pulmonary disease, hypertension and low birth weight of infants born to mothers who smoke [2]. Recent studies suggested that nicotine could have therapeutic applications in some neurodegenerative diseases like Alzheimer's [3].

[a] *Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos str., RO-400028, Cluj-Napoca, Romania.*
[*] *Corresponding author: ccimpoiu@chem.ubbcluj.ro*

The determination of total nicotine alkaloids is very important to toxicology and medicine and also for the tobacco industry because the quality of the product can be determined by its nicotine content. The nicotine was analyzed in different types of samples such as tobacco leaves, cigarettes, smokes, urine, hair, human serum, plasma etc. Many analytical methods have been developed and employed for nicotine determination in tobacco leaves and cigarettes, such as GC [4, 5], GC-MS [6, 7], TLC [8 - 10], HPLC [11, 12], HPLC-MS/MS [13], AAS [14], SFC–IMD [15], capillary electrophoresis [16, 17], radio immunoassay [18], spectrophotometric methods [19 - 23], circular dichroism spectropolarimetry [24], FTIR [25], voltametry [26] and recently flow injection with electrochemiluminescence detection [27]. All of these methods present a series of advantages and disadvantages.

The aim of this study is to evaluate and compare the nicotine content of twelve types of cigarette from popular brands available in the Romanian market. The analysis of nicotine in cigarettes is necessary due to the one global problem regarding counterfeit products trade. Some cigarettes contain more nicotine or the used tobacco can be of poor quality. In our original method different analytical parameters were optimized. This method is a good alternative to some of the reported expensive instrumental methods.

RESULTS AND DISCUSSION

Cigarettes contain various quantity of nicotine, depending on the brand, but only approximately 1 mg is actually absorbed in the human body [28]. The higher content of nicotine or tar content may cause serious health problems. Moreover, the nicotine addiction is related with higher risk for many kinds of diseases such as Alzheimer's, Parkinson's and even suicide [29, 30]. For these reasons the control of nicotine amount in tobacco products is obviously necessary.

On this line twelve cigarettes widely used and distributed in Romania were chosen for evaluation of nicotine content. For this aim a TLC method was developed and used.

After chromatographic runs using several mobile phases (**Table 1**) it can be concluded that the best separation was obtained using the mobile phase consisting in ethyl acetate - methanol - conc. ammonia - water 80:25:0.2:15.8, v/v/v/v (**Figure 1**).

The image of TLC separation obtained at 254nm (Figure 1a) clearly indicates the presence of different compounds in all of the analyzed extracts. The intensity of the bands separated on the chromatographic plate seem to be different in different sample, indicating that the concentration of these compounds depend on the type of cigarette. The chromatographic fingerprints of samples 3

and 5 present the most intense separated zones, therefore it can be admitted that they contain the highest amount of interest compound, including nicotine, that may correspond to one of these bands. Nevertheless, this image does not bring useful information about nicotine extracted from cigarettes, being a non-specific mode for identification of this compound.

Table 1. The tested mobile phases

No.	Solvent system	Composition (v/v/v/v)
1	Chloroform : methanol : acetic acid	11:8:1
2	Methanol : ammonia	200:3
3	Ethyl acetate : methanol : ammonia 0,1 M	80:25:16
4	Chloroform :methanol : ammonia	6:5:1
5	Dichloromethane : methanol : ammonia	83:15:2
6	**Ethyl acetate : methanol : ammonia : water**	**80:25:0.2:15.8**
7	Dichloromethane : methanol : ammonia : water	82:16:0.8:1.2
8	Acetonitrile : water	22:3

Thus, for certain identification of nicotine, the plate was sprayed with a specific color Dragendorff's reagent. This reagent usually forms an orange-red colored complex in the reaction with alkaloids. Consequently, the characteristic zone of nicotine appear as orange bands on a yellow background of derivatized chromatographic plate (**Figure 1b)**. The image of the TLC separation indicates that all analyzed samples contain nicotine.

The different intensity of nicotine characteristic bands show that its concentration is different in the analyzed extracts. Thus, samples 3 and 5 seem to contain the highest amount of nicotine, being followed by samples 6 and 11. The smallest amounts of nicotine seem to be contained in samples 4 and 1. In order to confirm that some samples contain different concentration of nicotine and to prove that some types of cigarettes contain the same concentration of this compound, the areas of the characteristic bands of nicotine were determined using ImageJ software. The areas were calculated from the image taken in UV light at 254nm because the sensibility of determination is higher than that in visible light.

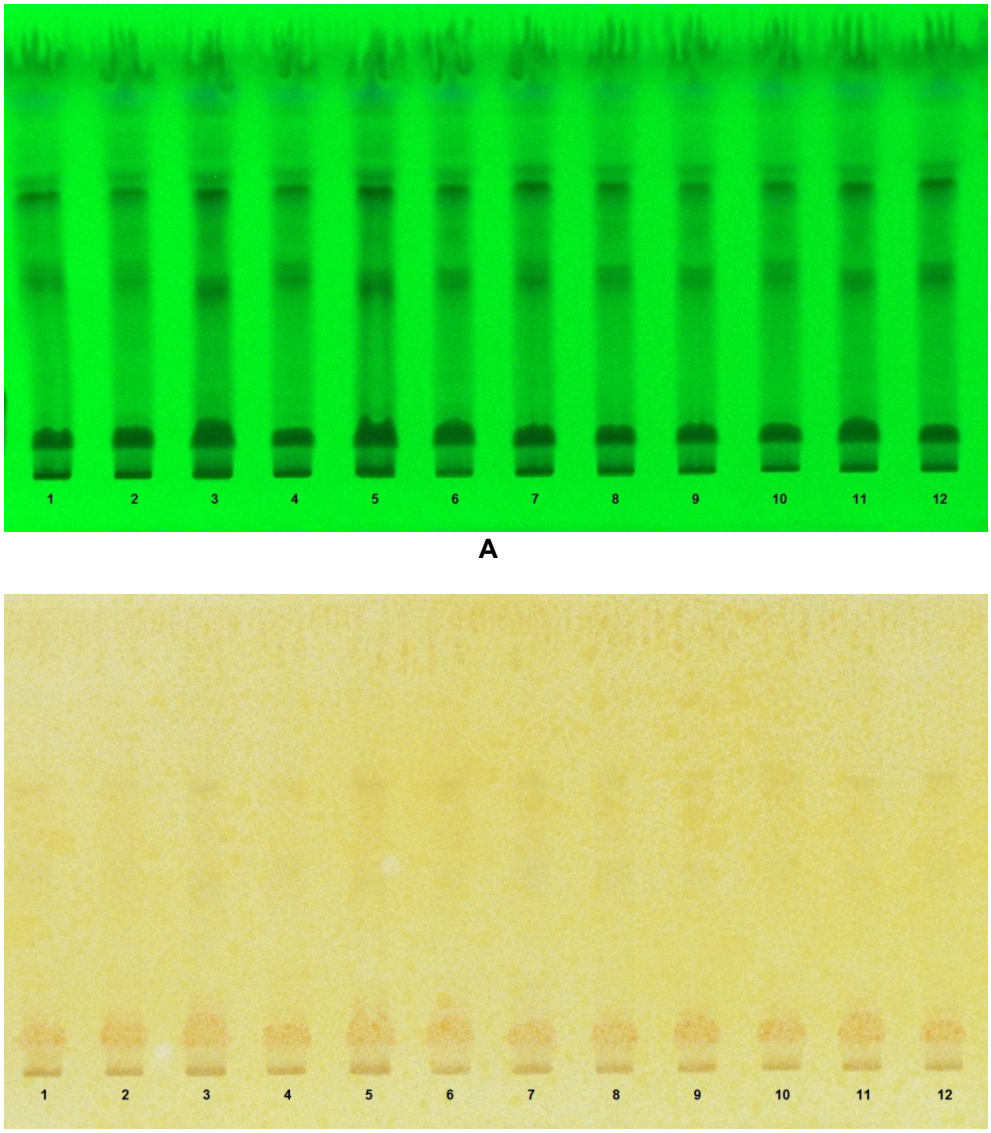

Figure 1. The TLC separation of nicotine from analyzed cigarettes:
A - UV light detection at 254nm; B - detection in visible light
after derivatization with Dragendorff's reagent.

The results (**Table 2**) show that samples 3 and 5 contain the same highest amounts of nicotine (label - 0.9mg nicotine/cigarette), followed by samples 6 and 11 (label - 0.8mg nicotine/cigarette), samples 2, 7 and 9 (label - 0.7mg nicotine/cigarette), samples 8 and 12 (label - 0.6mg nicotine/cigarette), sample 10 (label - 0.5 mg nicotine/cigarette), sample 4 (0.3mg nicotine/ cigarette), and the smallest determined area of nicotine band is that of sample 1 (label - 0.1mg nicotine/cigarette).

Table 2. The characteristics of analyzed cigarettes and the obtained area of nicotine bands

Sample	Cigarette Type	Producer	Nicotine (mg)	Area (AU)
1	Kent 1 (White)	British American Tobacco Group	0.1	3280
2	Kent 8 (Blue)	British American Tobacco Group	0.7	4145
3	Dunhill classic blend	British American Tobacco Group	0.9	5453
4	Kent 4 (Silver)	British American Tobacco Group	0.3	3519
5	Winchester	JT International Manufacturing	0.9	5481
6	Kent classic	British American Tobacco Group	0.8	4623
7	L&M red label	Philip Morris	0.7	4299
8	Kent convertibles (iSwitch)	British American Tobacco Group	0.6	3987
9	Viceroy red international blend	British American Tobacco Group	0.7	4199
10	Kent 6 (Spectra)	British American Tobacco Group	0.5	3779
11	Marlboro classic flavor	Philip Morris	0.8	4746
12	Pall Mall charcoal filter	British American Tobacco Group	0.6	3990

These semi-quantitative results are in concordance with the concentrations of nicotine expressed in mg of nicotine/cigarette, which are specified by the producers on the label of each type of cigarettes used in this study (**Table 2**). Moreover, a significant variation of nicotine content was noticed among the twelve investigated types of cigarettes (Figure 2), the highest amounts of nicotine (Kent 8 - sample 5) being almost duplicated compared to lowest nicotine content (Kent 1 - sample 1).

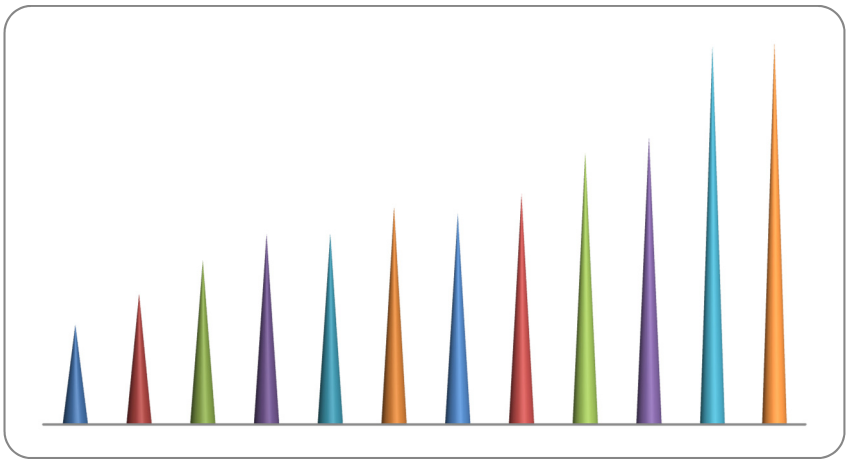

Figure 2. The areas variation depending on the type of cigarettes.

CONCLUSIONS

The developed method proved to be a powerful, low cost and simple tool for evaluation of nicotine in different sample. It allowed the simultaneous identification and semi-quantitative determination of nicotine from different types of cigarettes by TLC. Also, the determined areas of the characteristic bands of nicotine that are in keeping with the content of nicotine declared by producers enable to distinguish between cigarettes based on their content in nicotine. The method could be used for rapid detection of counterfeit cigarettes.

EXPERIMENTAL SECTION

Materials and reagents

All reagents and solvents used in the present research were of analytical grade and were purchased from Chimopar (Bucharest, Romania). The chromatographic plates were acquired from Merck (Darmstadt, Germany).

Twelve types of cigarettes from different producers were bought from tobacconist (**Table 2**).

Samples preparation

5mL of extraction solvent (ethanol-water 4:1, v/v) were added to 0.5g of tobacco from each type of cigarette. Extraction was performed by maceration at room temperature. After 10 days, the extracts were filtered and directly analyzed without any other treatments.

Chromatographic analysis

The chromatographic analysis was performed on silica gel F_{254} TLC aluminum sheets (20x10cm). Several mobile phase (**Table 1**) were tested, the chosen one being ethyl acetate - methanol - conc. ammonia - water 80:25:0.2:15.8, v/v/v/v. 10µL of each ethanolic extract were applied on the plate as 6 mm bands at 1.5cm from the low edge of the plate, with a rate of 80nL/s, using a semi-automatic applicator device (Linomat 5 - Camag, Muttenz, Switzerland) controlled by winCats software. The plates were developed at room temperature into pre-saturated (30 minutes) normal chromatographic twin trough chamber (Camag) to a distance of 80mm. Detection was performed under UV light at 254nm and under visible light after spraying with Dragendorff's reagent solution. Documentation of the plate was performed using a TLC vizualizer device (Digistore 2 - Camag) and the images were stored as jpeg. files.

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