

# Study of alkaloid (nicotine), in various smoking articles by thin layer chromatography

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## ABSTRACT

**Objective:** The present study deals with the finding of the nicotine content in various smoking articles available in the market by thin-layer chromatography (TLC) method. **Methods:** Various solvent systems were used to find the spots of alkaloid, nicotine, by TLC by standard procedures. **Results:** It was observed that in all the samples studied, the nicotine content was almost same as visualized by the resolution front values of each spot. **Conclusion:** It was concluded that although there are a number of smoking article available in the market, the nicotine content seems to be the same.

**KEY WORDS:** Alkaloid, Nicotine, Resolution front value, Solvent, Solvents, Thin-layer chromatography

## INTRODUCTION

Leaves of *Nicotiana tabacum* contain nicotine, 3-(1-methyl-2-pyrrolidinyl), pyridine which are colorless, pale yellow, and hygroscopic nature. Nicotine causes health issues such as elevated blood pressure and heart rate. Long period of tobacco use increased cancer rates, incidence of arterial disease, obstructive pulmonary disease, hypertension, and low birth weight of infants born to mothers who have smoking habit. Various studies show that nicotine has therapeutic applications in neurodegenerative diseases like Alzheimer's. Many analytical methods are used for the determination of the nicotine in the tobacco leaves and in smoking materials such as gas chromatography (GC), GC-mass spectrometry (MS), thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), HPLC-tandem mass spectrometry (MS/MS), (AAS) Atomic Absorption Spectroscopy, SFC-IMD (Supercritical Fluid Chromatography-Ion Mobility Detector) supercritical fluid chromatography-ion mobility detector, capillary electrophoresis, radioimmunoassay, spectrophotometric methods, circular dichroism spectropolarimeter, Fourier-transform infrared spectroscopy, voltammetry, and recently flow injection with electrochemiluminescence detection. Nicotine is

used for the treatment of inflammations, dyspepsia, dental caries, arthritis, gout, flatulence, bronchitis, scabies, asthma, rhinitis, and rheumatism. Nicotine is a liquid volatile colorless alkaloid with approximately 0.6–3% of the dry weight of tobacco. Worldwide, more than 3 million people currently die each year from smoking, and more than one-third have cardiovascular events that often determine permanent disability.<sup>[1-5]</sup>

In India, tobacco consumption continues to rise and a recent study of mortality associated with smoking in India estimates at least 930,000 adult deaths and that this would rise to over one million annually from 2010 onwards.

The major physiological effects of nicotine are summarized as follows:

- CNS stimulation with improvement of attention and memory
- Reduced hunger and body weight
- Increased metabolic rate
- Lipolysis, increased level of free fatty acid
- Increase in heart rate, cardiac output, and blood pressure
- Coronary vasoconstriction
- Relaxation of skeletal muscle.

Chromatography is a perfect analytical technique for the identification, isolation, and separation of compounds based on differences in affinity for stationary phase and mobile phase. In chromatography, the stationary phase is either solid, liquid, gel, or a solid-liquid

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mixture and the mobile phase could be liquid or gas. TLC is carried out by making thin layer of adsorbent materials; generally, silica gel and aluminum oxide are commonly used as adsorbent materials over the glass plate, plastic, etc. Within a short period of time, large number of sample can be analyzed simultaneously with the help of TLC technique, material which is non-volatile or having low volatility and which is not responding by liquid chromatography and gas chromatography, then for those kinds of material TLC are useful. Separation of substance is based on the solute and the mobile phase on the stationary phase. If the substance is more polar, resolution front (Rf) value getting more than that of less polar material. TLC is useful for both qualitative and quantitative analysis. Quantitative analysis can be carried out by measuring the intensity of the orange or brown color produced by the condensing iodine vapors on the spots.

The principle of chromatography is adsorption and partition, while mixture of substance is allowed to pass through stationary and mobile phase, a compound becomes separated according to their Rf.

$$R_f = \frac{\text{distance traveled by the sample salute/}}{\text{distance traveled by solvent front}}$$

Rf value is depended on some factors such as type of adsorbent, quantitative mixture of solvent, volatility, and temperature. If temperature is more than quick, evaporation will take place so better to keep running chamber away from the heat, sunlight, etc., thickness of the material, moisture content, running tank situation, and quantity of samples. TLC having variety of application such as TLC of amino acids pharmaceuticals and drugs identification, purity test and evaluating the concentration of active ingredients, preservative in drug, preparation process control in multipotent pharmaceutical formulation, qualitative analysis of alkaloid, in cosmetology, food analyses, petroleum product, aromatic compound isolation, molecular distillation, characterization of vitamin antibiotics, and definitions of drugs and inorganic ions. For screening and identification of many substances, planar chromatography is also used.

## MATERIALS AND METHODS

### Sample Collection and Preparation

Six types of market samples of smoking articles were collected from the market and labeled as M1, M2, M3, M4, M5, and M6. The tobacco content from each sample is separately taken and 15 g of each sample was extracted for nicotine using nicotine extraction solution, i.e., having ethyl acetate:methanol:ammonia:water for 24 h for complete extraction of alkaloid which is nicotine.

### TLC Plate Preparation and Running

- Silica gel and distilled water mixed to get thick slurry, then placed and spread over the unreactive career such a glass or plastic.
- Plates were dried.
- Plates were marked at bottom and top for spotting sample, to stop running of solvent beyond end of the slides marking done also at the top.
- Capillary tube dipped into respective test solution so that solution rises up in the tube.
- Prepared plate where briefly touched by the capillary tube at the start line so that the test solution get absorbed.
- Developing chamber saturation: Before running the TLC plate, saturation of the develop chamber is most important factor, without equilibration of the chamber, irregular running will take places resulting poor separation. Hence, need to saturation of the chamber and it is done by the placing of solvent of interest in the running chamber and allowed to stand for 30–60 min with a lid over the top of the chamber so that it becomes saturated with mobile phase solvent.
- Plates were placed at the developing chamber containing solvent of interest after sometime solvent front reached at the top near the marked line.
- Plate was taken out and dried to evaporate the solvent at the stationary phase.
- Visualization was carried out with iodine chamber.

## RESULTS AND DISCUSSION

Table 1 indicates the types of solvents used for TLC process. Table 2 shows the presence of alkaloid (nicotine). During analysis of various smoking article, it was observed that in all cases nicotine alkaloid presents, clear indication from the spots intensity of TLC plates. Table 3 shows the resolution font value of market

**Table 1: Various solvent system used in the study**

Solvent system	Ratio (V/V)
Acetonitrile:water	22:3
Chloroform:methanol:aceticacid	11:8:1
Methanol:ammonia	200:3
Ethyl acetate:methanol:ammonia 0.1 M	80:25:16
Chloroform:methanol:ammonia	6:5:1
Dichloromethane:methanol:ammonia	83:15:2
Ethyl acetate:methanol:ammonia:water	80:25:0.2:15.8
Ethyl acetate:methanol:water	12:35:3

**Table 2: The presence of alkaloid (nicotine)**

Sample	Presence (+)/Absent (-)
M1	+
M2	+
M3	+
M4	+
M5	+
M6	+

samples. Table 4 Relative rf values Alkaloid in different market samples of smoking articles. Figure 1 indicates the comparative values of Rf for each spot. From Tables 2 and 3, the Rf values indicating that there is no

significant difference among Rf values of all smoking articles. In all smoking articles directly indicating the presence of the nicotine, since the intensity of color is more and clearly spotted on the plates which also indicate the quantity of the alkaloid present of alkaloid in the smoking article. At the time analysis, there were two or more spots obtained among all spots only one spot having more brown color intensity and remaining are less intensity spot, but the important thing is that the more intensity spot more or less similar to each other for all plates and there was not too much variation of range among all plates. Two or more spots tell that in all market samples, alkaloid content is more and different types of alkaloid (or nicotine derivatives) are present during running the TLC plates, they were separated according to the polarity of molecules and depend on the molecular separation of molecule.

**Table 3: The resolution font value of market samples**

Alkaloid (nicotine) content in smoking article	
Sample	Resolution front (Rf)
M1	0.385, 0.769, 0.9846
M2	0.231, 0.815, 0.877, 1
M3	0.385, 0.8, 0.969
M4	0.279, 0.738, 1
M5	0.261, 0.862, 0.923, 0.985
M6	0.277, 0.815, 1
M7	0.815, 0.907, 1

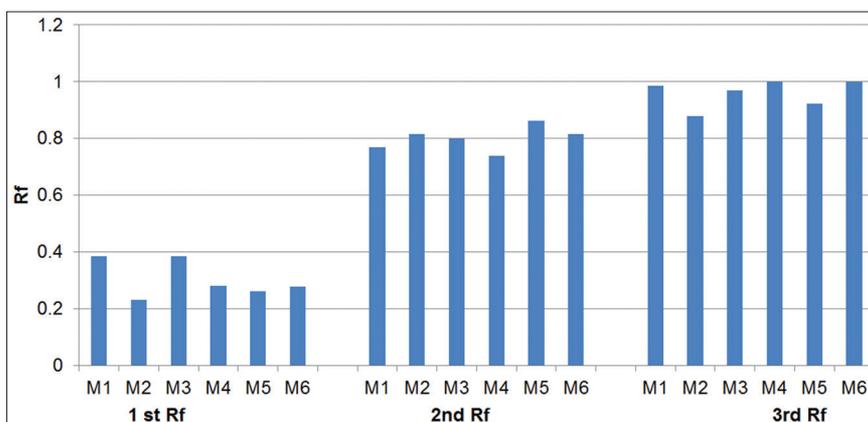
RF: Resolution front

**Table 4. Relative rf values alkaloid in different market samples of smoking articles**

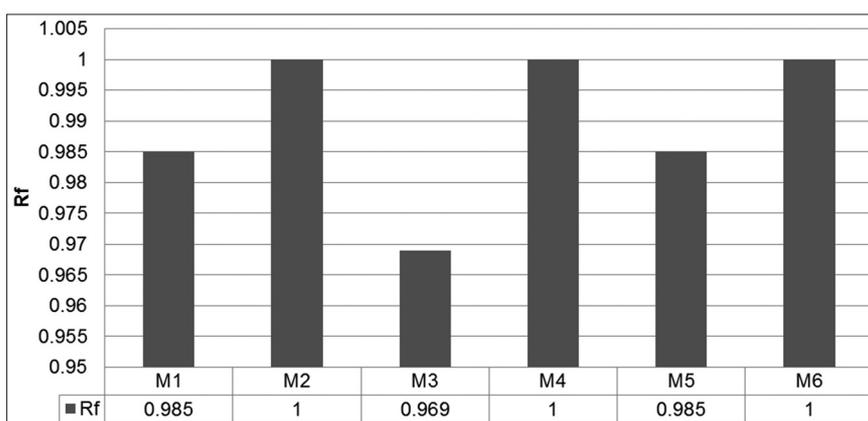
Alkaloid spot and Rf				
Sample	1 <sup>st</sup> Rf	2 <sup>nd</sup> Rf	3 <sup>rd</sup> Rf	4 <sup>th</sup> Rf
M1	0.385	0.769	0.985	-
M2	0.231	0.815	0.877	1
M3	0.385	0.8	0.969	-
M4	0.279	0.738	1	-
M5	0.261	0.862	0.923	0.985
M6	0.277	0.815	1	-

RF: Resolution front

From all spots of each plate, resolution font value was calculated and summarized in table form and graph also plotted to understand whether any special difference is indicating or not from various market samples. It was observed that in all plates, the Rf values range was nearer to the each other for all spots in all plates for all 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> Rf value and overall Rf values comparison from table and graph [Table 3 and Figures 1 and 2].



**Figure 1:** Indicates the comparative values of Rf for each spot



**Figure 2:** Overall comparison of various smoking samples

## CONCLUSION

Nicotine alkaloid presents in all smoking articles.

There is no significant difference between in market sample; they are using same tobacco materials.

Two or more spots found, brown color indicating intensity of quantity of nicotine alkaloid presence.

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