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Detection of active ingredients of Cannabis and Tobacco stuffed inside cigarette by two dimensional Thin Layer Chromatography

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ABSTRACT

In one case, some exhibits were received where the modus operandi was smoking of tobacco and *Cannabis* mixed together in cigarettes. Though not unusual but such modus operandi are not very common except for some parts in India. Smoking of tobacco along with *Cannabis* poses a double threat to the users as the toxicity and addiction increases manifold times. The detection of active ingredients of tobacco and cannabis by two dimensional thin layer chromatographic technique and the toxicological aspects are discussed in this paper.

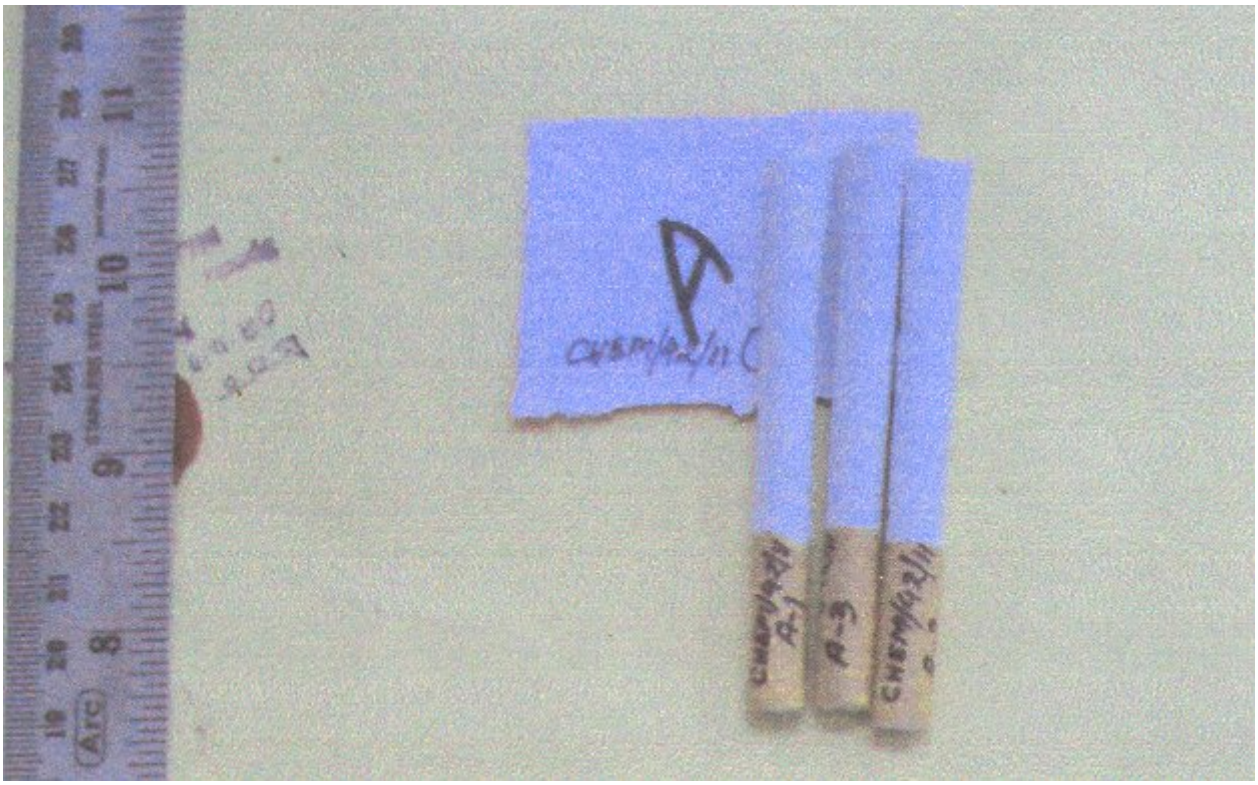
Keywords: Ganja, Cannabis, Tobacco, Thin layer chromatography, Toxicity

Introduction

The use of narcotics (plant origin) and tobacco are increasing even though statutory warning are printed on different products. Every packet of tobacco bears a statutory warning, but smoking has become very common among the younger generation to develop a feeling of manhood. And combined smoking of tobacco with cannabis gives a faster high and is gaining popularity due to the ease of modus operandi.

Brief history of the case : In one case, a small shopkeeper in town was secretly selling cigarettes stuffed with tobacco and cannabis dust to its customers and addicts. Based on secret information the investigating agencies seized some cigarettes, some small paper packets and some partially dried broken plant materials with characteristic smell. The seized samples were sent to the

laboratory for chemical examination and opinion, the owner was detained under NDPS Act. The exhibits received in the laboratory are shown in the Fig. 1.



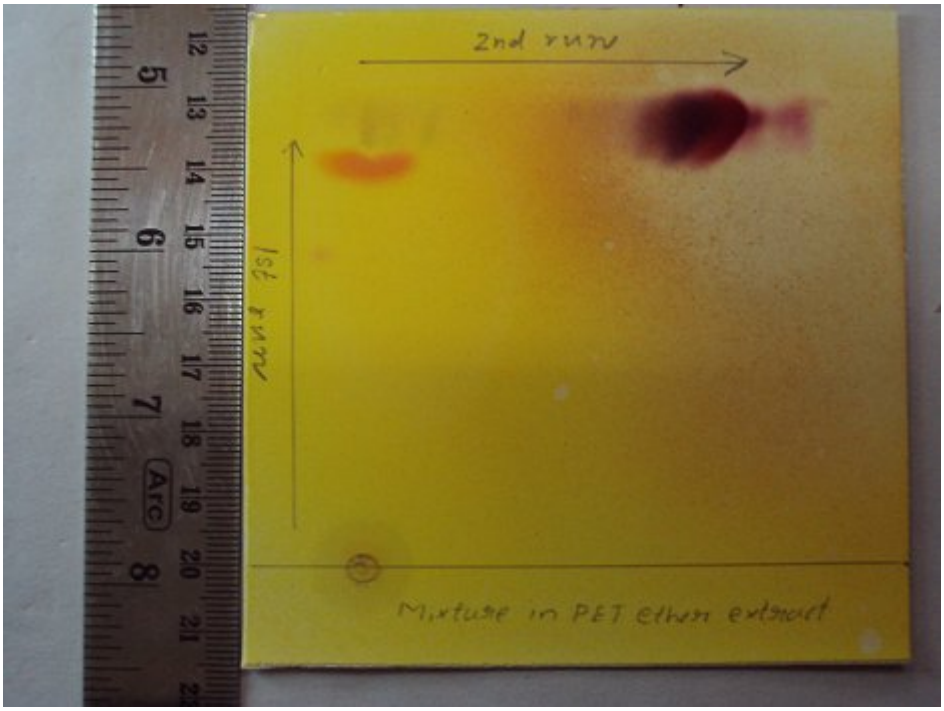
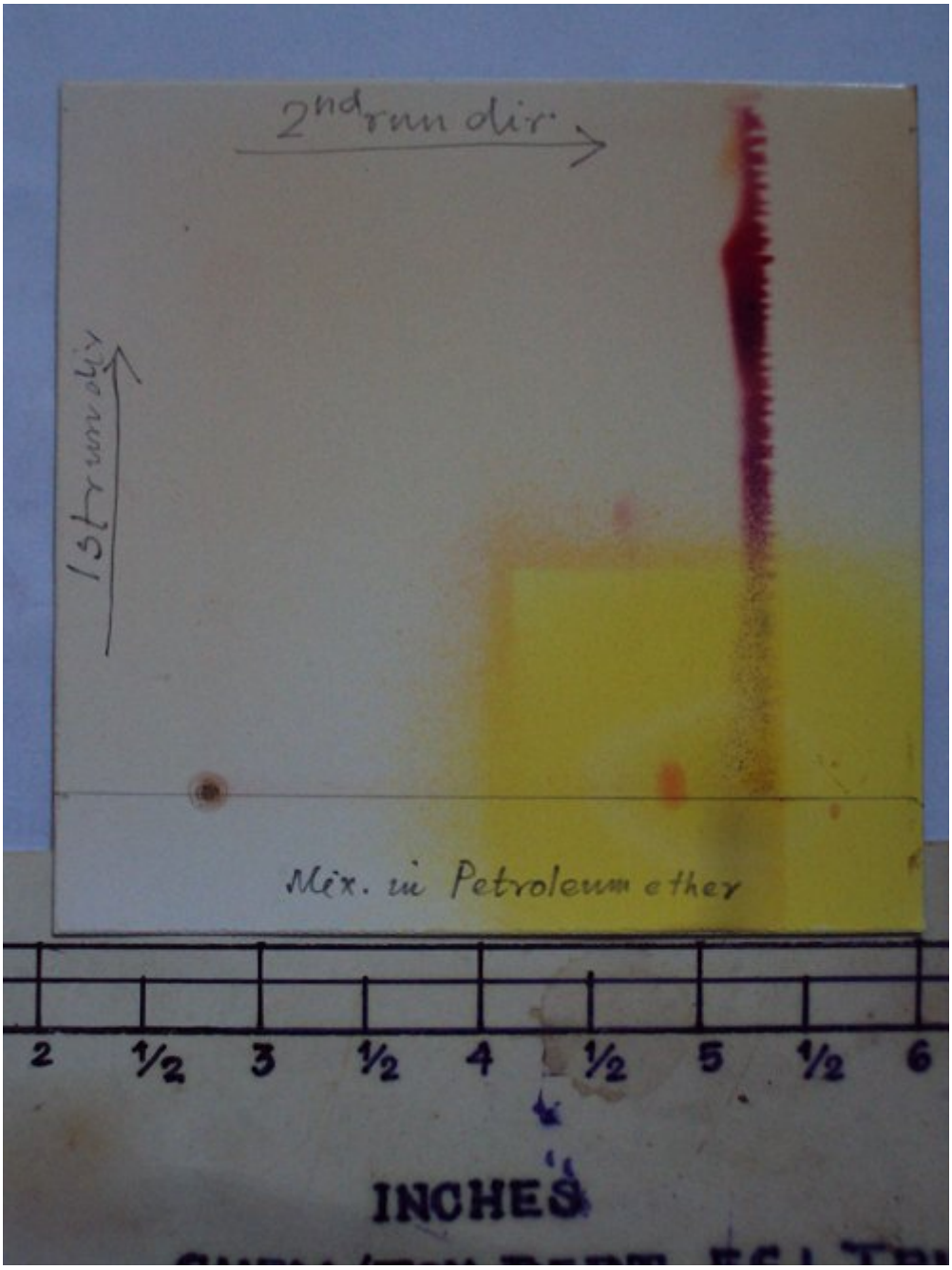


Fig. 1. Cigarette samples with cannabis dust received for chemical examination. **Fig. 2.** TLC photograph of cannabis and tobacco extracted in petroleum ether followed by TLC in 1st solvent system then by second solvent system.


2nd run dir.
1st run dir.
Mix. in Petroleum ether
2
½
3
½
4
½
5
½
6
INCHES

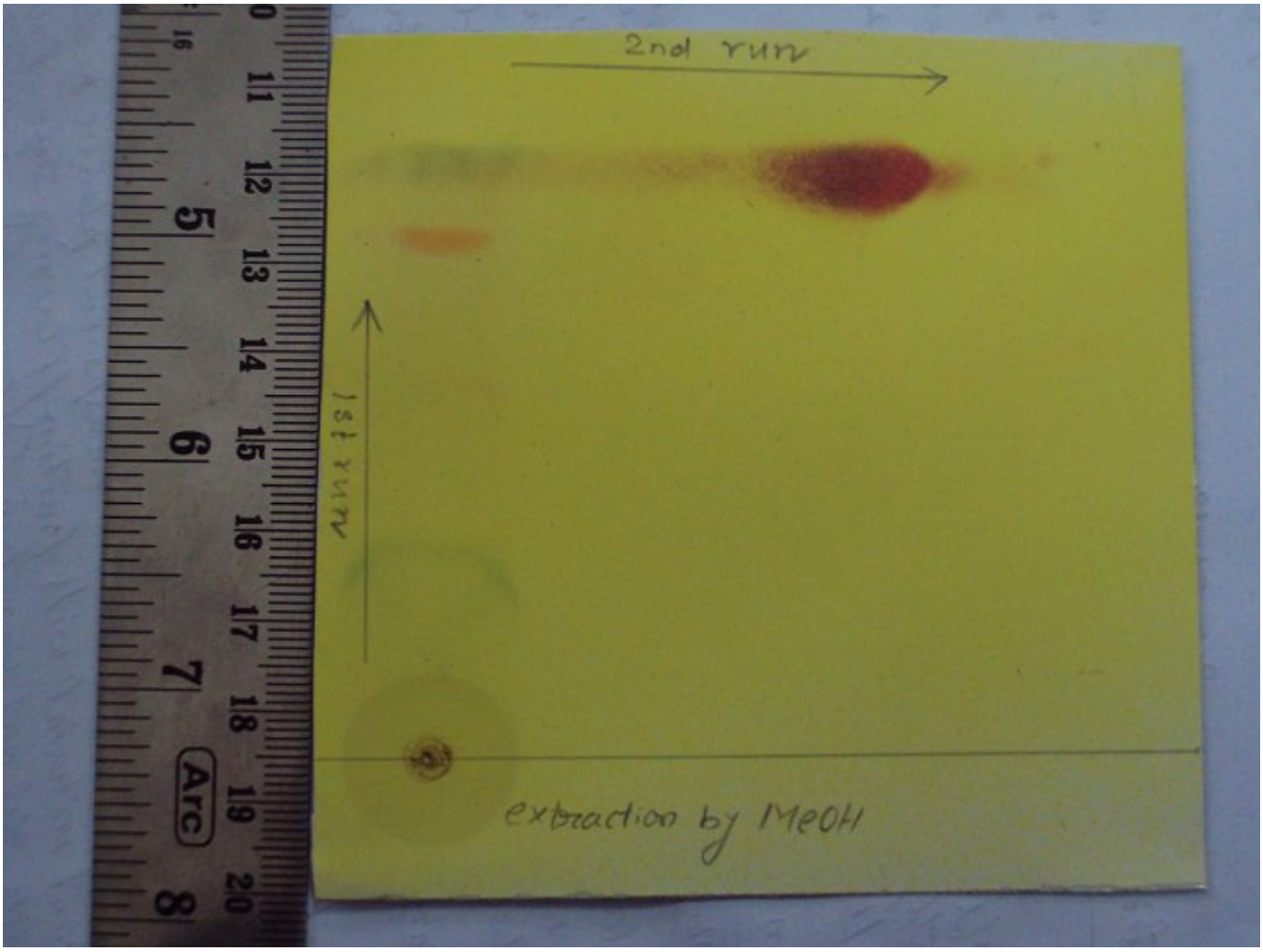


Fig. 3. TLC photograph of cannabis and tobacco extracted in Petroleum ether followed by TLC in 2^{nd} solvent system then by 1^{st} solvent system. **Fig. 4.** TLC photograph of cannabis and tobacco extracted in Methanol followed by TLC in 1^{st} solvent system then by 2^{nd} solvent system.

1^{st} solvent system system.

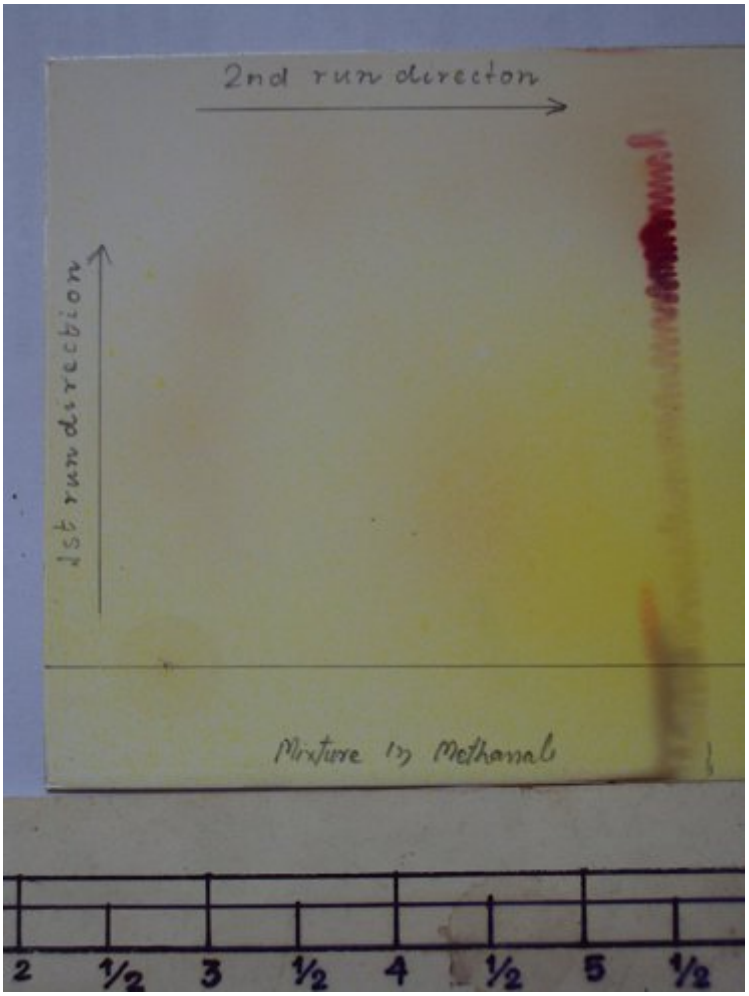
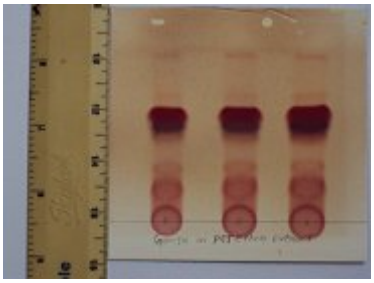


Fig. 5. TLC photograph of cannabis and tobacco extracted in Methanol followed by TLC in 2nd solvent system then by 1st solvent system



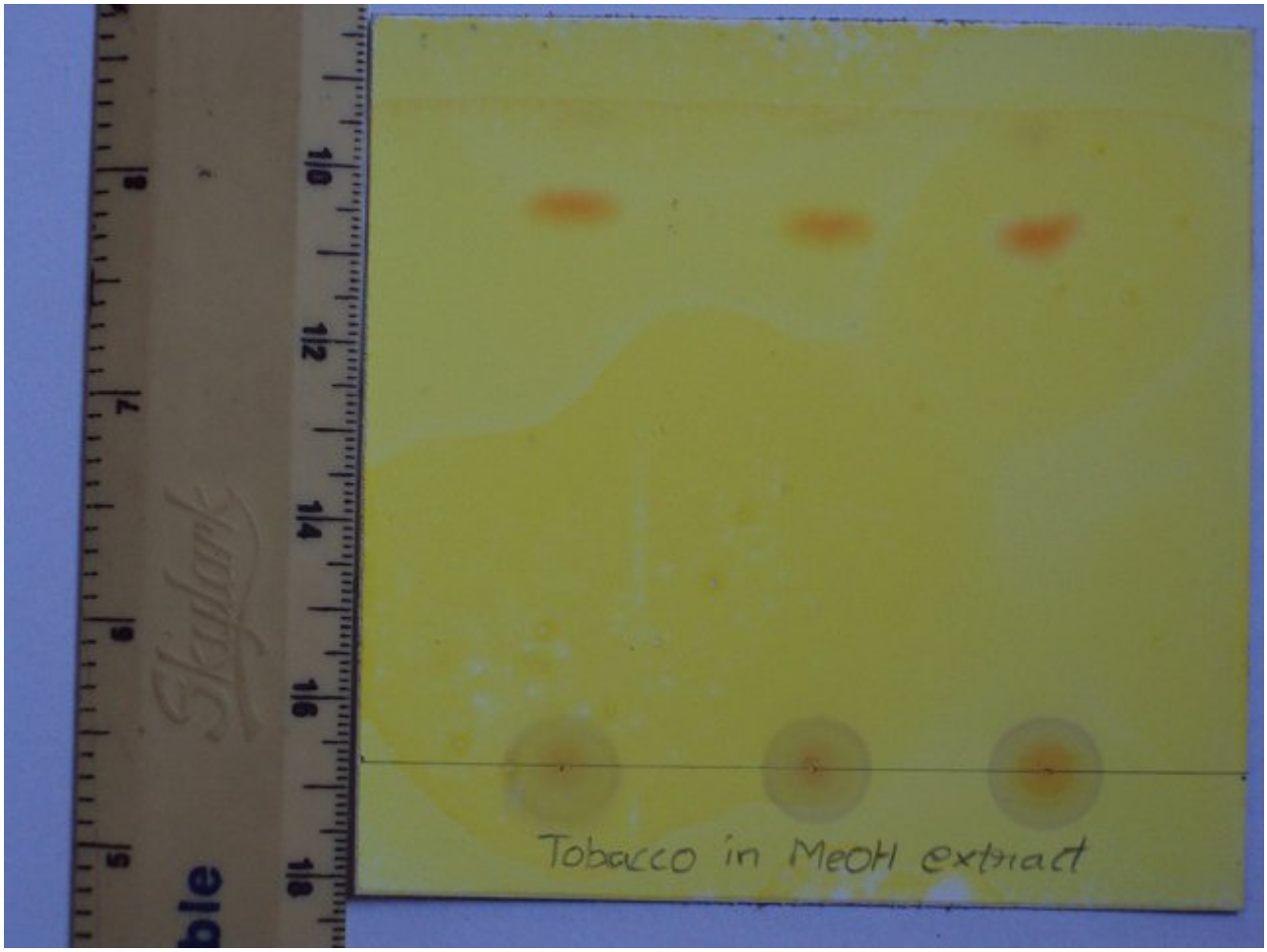


Fig. 6. TLC photograph of only Cannabis extract in Petroleum ether. **Fig. 7.** TLC Photograph of only Tobacco extract in methanol.

Observations

Orange yellow colour spot -- for nicotine

Purple red colour spot -- for cannabinoids

Materials and methodology: The exhibits received in the laboratory were taken up for chemical examination as per procedure laid down by UNDCP manual. Except the stuffed cigarettes, the rest exhibits as preliminary tests indicated that they were not mixtures, the samples were extracted by petroleum ether and the extract was taken up for thin layer chromatographic study as per the following procedure:

Solvent system: Petroleum ether: Diethyl ether(8:2)

Visualization reagent: UV 254 nm/ Fast Blue B salt in Methanol

TLC plates: 20x20 cm, Silica gel $60F_{254}$

The cannabinoids are easily soluble in most organic solvents like Petroleum ether, n-Hexane, Toluene, Chloroform, Methanol. Solvent mixture like Methanol: Chloroform (9:1) are also equally suitable compound for the extraction purpose. But n-Hexane and Petroleum ether gives a relatively clean extract, and extracts only the neutral cannabinoids but the other solvents or solvent combinations give qualitative extraction of cannabinoic acid as well. But the determination of the major cannabinoids namely Tetra hydro cannabinol, Cannabinol and Cannabidiol are usually best achieved by extraction with Petroleum ether(Fig. 6). The stand- off technique and as per records the suitable solvent for extraction of active ingredients of tobacco is Methanol and it has been reported that tobacco extraction by Methanol gives a good yield of the active ingredients (Fig. 7) but is likely to extract the Cannabinoic acids as well. Therefore to conduct a study on the reproducible detection of cannabinoids and the nicotine from the cigarettes samples, the mixed samples collected from inside the stuffed cigarette were extracted both by Petroleum ether and Methanol separately.

Analytical section: Keeping in view of the available resource of the laboratory, Thin layer chromatography was selected as the analytical technique. A single run technique of the extracts of *Cannabis* and nicotine taken together either in Petroleum ether or in Methanol was found not to be effective. In this case the nicotine was found to be mostly masked and remain non identifiable in particular after spray of Fast Blue B (in Methanol). Nicotine develops a small spot in comparison to the cannabinoids and until the cannabinoids and Nicotine are separated to a considerable distance, it is difficult to obtain a reproducible result. In such case an effective separation can only be achieved by two dimensional Thin layer chromatography technique with a marked variation in the polarity of the solvents for obtaining significant result in analysis and separation. A two dimensional thin layer chromatographic system with solvents systems having a marked difference in polarity were selected. The sample, namely the mixture of nicotine and *Cannabis* were extracted both in Petroleum ether and Methanol separately. Both the extracts were eluted in a TLC chamber consecutively by the below mentioned solvent systems.

First solvent system - Ethyl acetate : Methanol :conc. Ammonia (42.5:5:2.5)

Second solvent system - Petroleum ether: Diethyl ether (8:2)

Visualization reagents – 1. UV 254 nm

2. Draggendorf’s reagent

3. Fast Blue B in Methanol.

Initially after completion of each run the occurrence of the spots were noted under UV-254, air dried the plate, rotated by 90^0 and carried out the second run. Samples in a two dimensional chromatography study were spotted at one corner of the plate, developed in the second solvent system. On completion of the run, the plates were subjected to spray of chromogenic reagents for development of characteristic colors. A parallel chromatogram with the individual samples were carried out.

Discussion

The mixture of ganja and tobacco extracted in Methanol and Petroleum ether is identified by a two dimensional Thin layer chromatographic system. The active ingredients of tobacco are best extracted in Methanol whereas the neutral cannabinoids along with other cannabinoic acid are

also extracted by Methanol. The TLC of the petroleum ether extract was done by the first solvent system i.e., Ethyl acetate : Methanol: conc. Ammonia , then carried out the TLC by the second solvent system i.e., Petroleum ether: Diethyl ether. The finished chromatogram is a two dimensional array of spots. Nicotine gives a orange yellow colour in Draggendorfs' whereas the active ingredients of *Cannabis* are identified by their characteristic purple red coloration (Fig- 2 & 4). In both the plates cannabinoids and nicotine were noticed to be eluted along with the first solvent system but with the second solvent system nicotine remained stationed at the base and cannabinoids were eluted. There was separation of the cannabinoids and nicotine at a considerable distance and distinct colour development were noticed for both nicotine and cannabinoids.

A reverse study was done with the same extract, first elution by Petroleum ether: Di ethyl ether (2^{nd} solvent system) then by Ethyl acetate : Methanol: conc. c. Ammonia(1^{st} solvent system) followed by application of spray reagents (Fig.-3 & 5). In the reverse study , it was observed that the R_f value of the active ingredients of nicotine was little above the base line as the second solvent system does not raise the nicotine spot significantly but in the subsequent run it was observed that the first solvent system raised the nicotine to a significant height. The orange yellow spot of the nicotine was stationed almost at the base line which was raised along the base line at a significant level during the second run. However the separation of the cannabiniods were not pronounced in the reverse study. The study conducted with the methanol extract are shown in fig 4 & 5 . It was observed that the two dimensional study with the petroleum ether extract gave a better separation in comparison to the methanol extract.

The mechanism of retention differs with the change in the solvent system, thus making it possible to separate compounds that are indistinguishable by one dimensional thin layer chromatography. In the study conducted, the two dimensional run first by solvent system -1 and then by solvent system-2 gives sharp and pronounced separation for both *Cannabis* and tobacco extract and it is best to extract the samples in Petroleum ether for reproducible results. And in comparison to the reverse run, the TLC with the first system followed by the second system yielded effective results. Ganja, contraband item under ND&PS act is referred to forensic laboratory for chemical examination and opinion. Both tobacco and cannabis are herbal products and a single run in a single solvent system doesn't give satisfactory separation. The two dimensional TLC system having a marked variation in polarity as proposed, separates the ingredients of tobacco and ganja to considerable distance thus reproducible result can be obtained for conclusive identification of nicotine and the Cannabinoids. Such two dimensional TLC studies find their applications in separation of other biological components also.

Toxicity of tobacco and cannabis : The smoking of cannabis with tobacco exposes to increased threat to the user. When a cigarette is lit and inhaled, the smoker is exposed to both gaseous and particulate phase of cigarette smoke. The usual nicotine content in a cigarette varies from 15 to 20 mg, whereas certain countries produce cigarette having higher amount of nicotine. The major physiological effects of nicotine are summarized below-

1. CNS stimulation with improvement of attention and memory
2. Reduced hunger and body weight
3. Increased metabolic rate
4. Lypolysis, increased level of free fatty acid

5. Increase in heart rate, cardiac output and blood pressure
6. Coronary vaso-constriction
7. Relaxation of skeletal muscle.

In addition to addiction nicotine smoking exposes to As, CO, HCN, Ni, Cd etc poisoning. It is already proven that inhalation of tobacco in any form causes cancer. In raw form, cannabis commonly known as ganja is smoked in paper or home made cigarettes. Sometimes it is also eaten directly or mixed with food. Though there are evidence of ganja being described in several Chinese and Indian text about its use in medicinal purpose, but smoking or eating raw cannabis can cause a number of adverse effect like feeling of euphoria, short term memory loss, increased difficulty in completing complex task, occurrence of change in perception of time and space, inability to concentrate in studies, depression, paranoia and hallucination. Though instances of death are rare but some have indicated that in an adult human LD_{50} of Tetrahydrocannabinol(THC) toxicity would be between 50-86 g in an 68 kg/ 70 kg female and male respectively. Such studies are made on the animal studies, however it is worth mentioning that THC receptor distribution in rat CNS is different from that of humans, and as such there may be a variable toxicity in humans based on animal studies.

According to many researchers marijuana contains known carcinogens or chemicals that can cause cancer. Using a new high sensitive technique, scientists from Europe have reported that *Cannabis* smoking damages the genetic material in DNA in such a way that continued use increase the risk of cancer, toxic substances present in tobacco smoke damage DNA increasing the rate of lung and other cancer. Moreover, it is great concern about the toxicity of acetaldehyde present in both tobacco and *Cannabis*.

As per study nicotine is most heavily addictive drug in United States and also concurrent in almost in all other countries. Since beginning of 20^{th} century, cigarette smoking is the most popular method of taking nicotine. Tobacco in its different form like cigar, pipe, snuff, bidi, khaini(tha last two especially in India) are addictive and nicotine, (an alkaloid $C_{10}H_{14}N_2$) found along with small quantities of nicotimine, nicoteine and nicotelline is the responsible factor for deleterious affect.

The combination of a narcotic drug namely *Cannabis* with tobacco not only increase addiction but develops drug dependence. Compared to a normal cigarette, a cigarette with *Cannabis* costs higher and with the increase of drug tolerance, there is a continued increase in drug requirement *Cannabis* high called “stonned” makes many users feel relaxed and talkative along with other pleasurable sensation like mood lift, relaxation altered senses etc. which cannot be achieved by tobacco alone. This factor propels the user to move towards a combined usage. Directly inhaled *Cannabis* smoke contained as much as twenty times more ammonia and five times more HCN as compared to tobacco smoke. The combined effect can be easily guessed thereafter.

Mostly laboratories rely heavily on Thin Layer Chromatographic system, being the most cost effective and can be managed by a laboratory technician under the supervision of the laboratory manager. This type of two dimensional system can conclusively help a forensic chemist confirm the presence of tobacco and cannabis in a same exhibit as a effective, fast, economic and reproducible methodology and thus helping the criminal justice delivery system.

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