Extraction and High Purification of Nicotine from Iraqi Tobacco Leaf Formanufacturing, Pharmaceutical, and Medicinal Uses

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Abstract

Bachground: Nicotine is highly addictive plant derived alkaloid and the most important species in human use today is Nicotianatabacum. There are direct health effects of chronic nicotine exposure. Even in low doses, nicotine causes vasoconstriction and other cardiovascular effects related to catecholamine release and promote angiogenesis, neuroteratogenicity, and possibly some cancers. **Methods:** A preliminary investigation to analyze the nicotine contained in Iraqi tobacco leaves was carried out using gas chromatography-mass spectrometry (GC-MS). Nicotine is an alkaloid, and alkali methanol and Lipophilic solventsystem methods (LSS) have been extracted and determined by GC-MS from tobacco leaves. **Results**: The detection limit for nicotine was for non-selective monitoring at the ppm level and for selective detection at the nanogram level. This is a simple method of thin layer chromatography (TLC) and chromatography mass spectrometry (GC-MS) for the tobacco leave analysis of nicotine. The final purity of nicotine is 99%. **Conclusion :**the methods which used in this study gave very high purity of nicotineafter converting the crude nicotine to its esters.

Key words*: Nicotine , Iraqi tobacco, Lipophilic solvent, TLC, GC-MS chromatography.*

Introduction

Nicotine is the predominant alkaloid in Tobacco plant (*Nicotiana. tabacum*) represent above 90% from total alkaloid[1].In the recent decade there are increasingly demanded on it for industrial uses as one of the most important component in E- cigarette flavors and nicotine gum. In the pharmaceutical and medicinal fields using as a curative agent against some of psychotic disorders such as Schizophrenia, degenerative disease such Alzheimer[2]. The aqueous two-phase system and solvent reverse extraction by using isopropyl alcohol/ $(NH_4)_2SO_4$. The recovery rate of nicotine was 96.1% with a purity of above 99% when optimal conditions were used [$(NH_4)_2SO_4$ 25%, pH 9, temperature 35°C, isopropyl alcohol 5 mL[3]. Marked solubility differences of nicotine in the ionic liquids [C(2)mim][NTf(2)], [C(2) mim][EtOSO(3)], and [C(n)mim]Cl, 6 <or= n <or= 10, are observed through the analysis of the corresponding phase diagrams. These show the potential of commonly used ionic liquids to extract and purify this important compound. From a fundamental standpoint, the generally enhanced solubility of nicotine in these ionic liquids as compared to that of aromatic and aliphatic hydrocarbons can be assignedto the presence of the aromatic pyridine ring and the large aliphatic N-methyl-

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pyrrolidine residue.[4] The molecular genetics of tobacco alkaloids have not only provided plant biologists with insights into the mechanisms underlying the synthesis and accumulation of this important class of plant alkaloids, they have also yielded tools and strategies for modifying the tobacco alkaloid composition in a manner that can result in changing the levels of nicotine within the leaf, or reducing the levels of a potent carcinogenic tobacco-specific nitrosamine (TSNA).[5]The Chemical components of nicotine got the general formula $C_{10}H_{14}N_2$, with molecular weight 162 Daltons [6], and the regular name pyridine 3-(1-methyl-2-pyrrolidinyl), maximum absorption in 254nM, its heterocyclic alkaloids have different tow rings (pyridine) and (pyrrolidine)[7] the two nitrogen in pyridine and pyrrolidine rings capable of being ionized :N-pyridine pKa =3.4, N-methylpyrrolidinepKa =8.1[8]. So, the behavior of nicotine is pH dependence, the entire nicotine soluble in lipophilic solvent in pH above 9 and not soluble in hydrophilic solvent, in pH =2 or less its miscible in the water at 25ºC or less[9].Dalton*et al*.,[10] found that most regarded of nicotine derivatives is its esters that have many uses because it have less harsh test in mouth or burning feel on skin and in I.V injection. Physically nicotine is the oily liquid, colorless or pale yellow solution, turn to brown whenexposure to air or light, fish-like odor when warm with density =1.000925 at 25ºC and Boiling point (B.P) = 247ºCatatmosphere pressure.[7]. The aim of this study is to purify the nicotine in high grade and prepare the salt of nicotine that used in industrial, pharmaceutical and medicinal fields.

Materials and Methods

The Iraqi tobacco leafs is purchased from local market, dried in the oven on 40ºC for 60 min, crushed by electric grinder to fine powder. The powder (500gm) potted into closable glass container, add 500ml of D.W thin NaOH crystals was added till reached the pH to 13.35,heated in water bath to 80c for 20 min, cooled to 50c,add ethanol 99% 4:1 ethanol: mixture reheated to 60c for 120 min let to cool to the 25ºC [11] , added chloroform in ratio 1:4,adjusted the pH to 1.0 by H_2SO_4 0.5 M, stirred for 60 min at 25ºC, tow layer are preform, keep the mixture in [-5ºC] overnight,separate by separation funnel took the ethanol layer and rewashed again by 1:4 by chloroform under same conditions, took the ethanol layer and adjusted the pH to 13.35 by NaOH solution 0.5 M added chloroform solvent in 1:4 ratio and stirred the mixture 60 min at 60 ºC in closed container thin separated, discarded the ethanol layer and took the chloroform layer rewashed by ethanol 75% pH 8.5 under the same conditions, took chloroform layer and evaporate the solvent by rotary evaporator. The pale yellow liquid obtained turn to brown yellow very thick slurry after exposure to air, this substance called crude nicotine

Preparation of nicotine esters (Nicotine bitartarate salt) :To prepare nicotine bitartarate mix approximately 1:3 mol/mol crude nicotine: tartaric acid.[12]. where : Molecular weight of nicotine is 162.23g/mol, so 8.1g =0.05 mol.Molecular weight of tartaric acid is 150.087 g/mol. so 7.505g= 0.5 mol[12,13].

Dissolved 7.505 g ×3=22.51g of tartaric acid in 100 ml cold methanol then added the 8.1g of nicotine and stirred the white crystals was observed the solution was potted in refrigerator at 4ºC overnight, collected the crystals by filter paper, washed the crystals by 50 ml methanol then 50 ml chloroform, this crystals represent nicotine bitartarate.Re-covered nicotine from nicotine bitartarate .with some modification. 20g salt dissolved into 100 ml ethanol added 0.5 M NaOH till the pH reached to 13.35 heated the mixture to 65 for10 min, added 100 ml of chloroform adjusted the pH to 13.35 and stirred with heat 30 min at 60 ºC add a drop of distill water until separated the two layers took chloroform layer and dried, the yellow slurry material represent the pure nicotine.[9].

Results and Discussion

The final material was examined by several testsfirst by preliminary screening by Wagner's and Mayer's reagent gave the result positive, Second by TLC 20×5 cm plate was used, system solvent was methanol: ammonia 200:3* , dyeing by iodine vapor , the RF=56.8 this result was very near compared to RF =57 (Figure 1)[13, 15]. Third test by using GC-MS test to identifying the identity of

substance on a specimen, the 99% of area mass was obtained to the nicotine component in specimen, this ratio is represent the purity of nicotine.

The solvent system, pH and temperature and time is a critical points in extraction efficiency of nicotine, chloroform - aqueous system have a good extraction ratio because the highest distribution coefficient between the two solvents, nicotine trend to dissolved in aqueous solvent whenever pH 3 or less and temp 25 or less than, vice versa nicotine dissolved in organic solvent at pH above 9 or higher and temp above 25°C. The purity of nicotine rose from 16.43% to 99% after converting crude nicotine to its esters (nicotine bitartarate)[16]. Therefore, because of the capacity of nicotine and few substances under the above conditions to make crystal esters. Nevertheless, all compounds (hydrophilic and lipophilic) seeded by wash only leave filter paper to arrest nicotine salts. This technique can be adopted to purify nicotine at least from Iraqi Tobacco, based on the above findings.

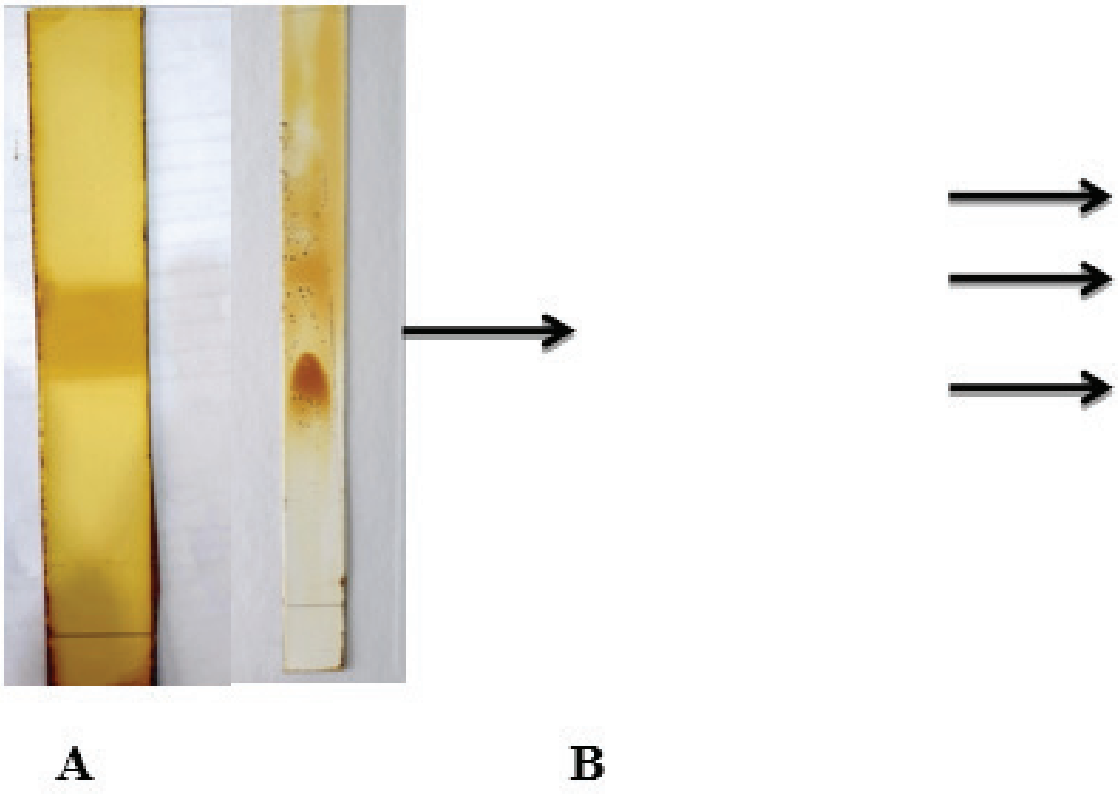

Figure (1): Thin layer chromatography plates of nicotine compound using solvent system

(methanol : ammonia (200: 3) . A: pure nicotine B: crude nicotine .

The used of alkali material not only toincrease pH value in extraction and recovery of nicotine, but return to capacity of this material with heat to break down the forms of nicotine esters (most nicotine contained in esters forms in plane tissue) and release nicotine in free form, the time, alkali concentration and temperatureare so important in nicotine extraction and yield due to nicotine degradation.[17,18] (Figure 2).

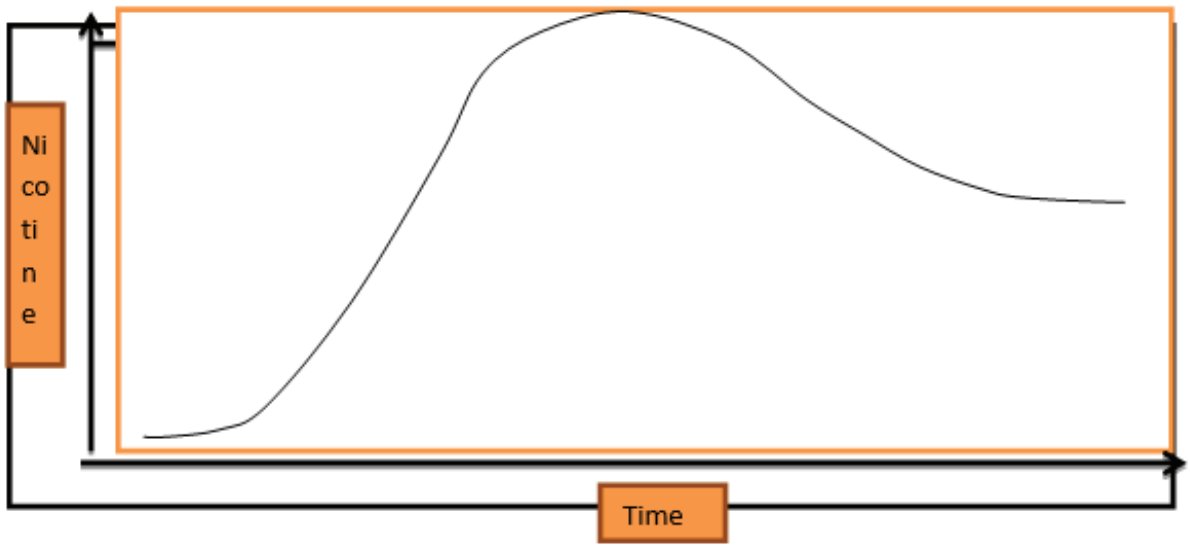

Figure 2: Critical points in extraction of nicotine: Alkali concentration, Time and Temperature.

Table 1 : GC-MS analysis of crude nicotine.

GC-MS Column Name		Agilent 19091S-433UI			
Peak NO.	**Retention Time**	**AREA %**	**Compound Name**	**REF**	**CAS #**
1	12.435	16.43%	S- pyridine 3-(1-methyl-2-pyrrolidinyl)	32085	000054-11-5
2	16.341	4.45%	Dimethyl-3-Butene-1,2-diol, 1-(2-furyl)-2,3 Octacosyl acetate 1-Heptacosanol	46869 227661 210517	019757-51-8 018206-97-8 002004-39-9
3	25.130	5.92%	Methoxyacetic acid, 2-tetradecyl ester Tetratetracontane Heptacosane, 1-chloro ethan	132964 241527 217413	1000282-04-8 007098-22-8 062016-79-9
4	26.428	15.91%	Tetratetracontane 2-tetradecyl Methoxyacetic acid ester Tritetracontane	241527 132964 241174	007098-22-8 1000282-04-8 007098-21-7
5	27.557	14.65%	Octadecane, 1-chloro-ethan Tritetracontane Methoxyacetic acid, 2-tetradecyl ester	134594 241174 132964	003386-33-2 007098-21-7 1000282-04-8
6	28.561	11.22%	1-bromo Octadecane Tritetracontane Hexadecane, 1-bromo	170753 241174 147852	000112-89-0 007098-21-7 000112-82-3
7	29.483	5.96%	tert-Hexadecanethiol Oxalic acid, dodecyl propyl ester Hexadecane, 1-bromo	109272 144569 147852	025360-09-2 1000309-26-5 000112-82-3

***Cont...* Table 1 : GC-MS analysis of crude nicotine.**

8	30.051	1.06%	13-Octadecenal, (Z)- Butyl 9-octadecenoate or 9-18:1 6-Nitroundec-5-ene	115867 175503 60376	058594-45-9 1000336-74-7 1000192-40-3
9	30.361	3.64%	Hexadecanoic acid Oxalic acid Octadecane	197985 203464 141404	157336-02-2 1000309-24-5 000930-02-9
10	31.163	9.82%	Erucic acid Oxirane Tetradecanal	175491 94324 71300	000112-86-7 007320-37-8 000124-25-4
11	31.714	10.93%	cis-Vaccenic acid cis-13-Octadecenoic acid Erucic acid	129339 129347 175491	000506-17-2 013126-39-1
TOTAL	32 Min	100%	31 COMPOUNDS	----	------

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Nicotine purity rose from 16.43% to 99% after converting the crude nicotine to its esters, According to figure 3 and 4 (nicotine bitartarate). Therefore, because of the capacity of nicotine and few substances under the above conditions to make crystal esters. Nevertheless all substance (hydrophilic & lipophilic) seeded by wash leave only filter paper to arrest crystals. Therefore in order to obtain the high purity of nicotine, the pH –dependent inversion solubility of nicotine from polar solvent is not necessary to obtain 99 percent purity of nicotine.

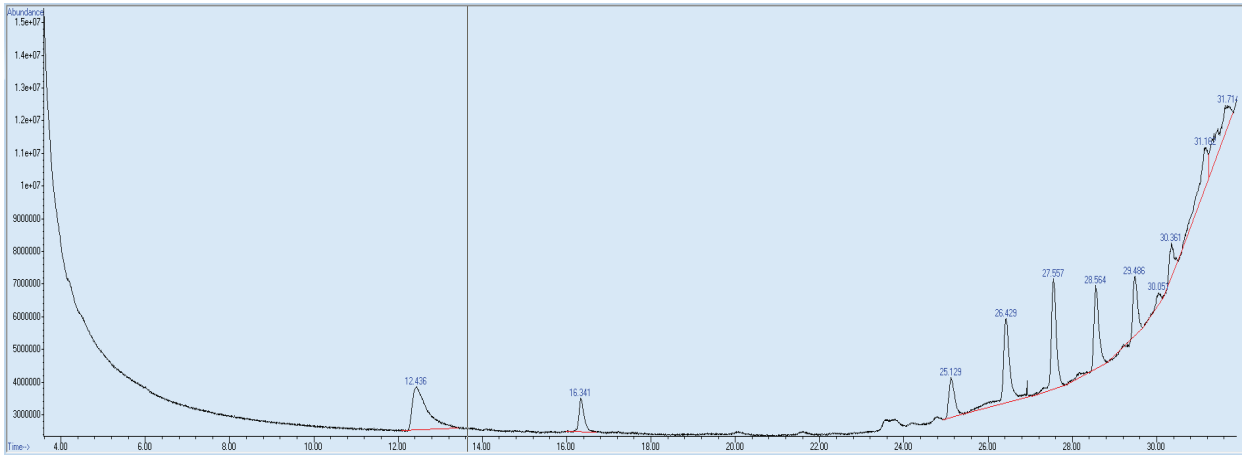

Figure (3): crude nicotine GC-MS assay nicotine partial purity just 16.4%.

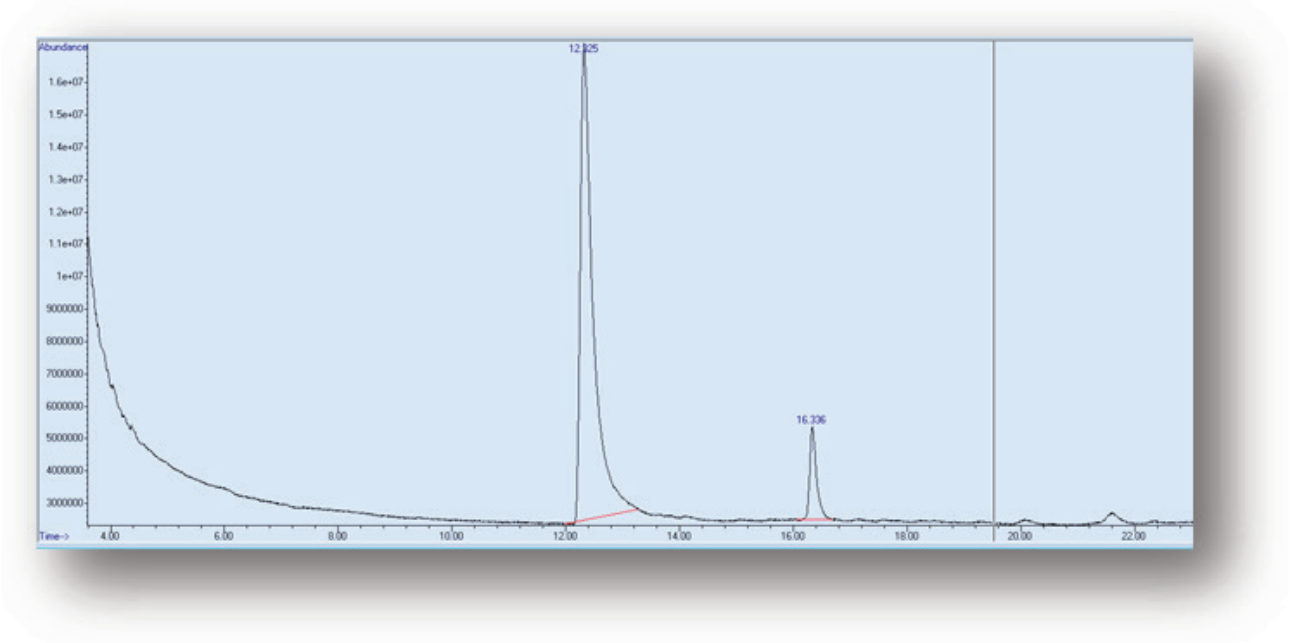

Figure (4) : Pure nicotine GC-MS assay the purity of nicotine reached .

Table 2 : GC-MS analysis of pure nicotine.

GC-MS Column Name		**Agilent 19091S-433UI**			
Peak NO.	**Retention Time**	**AREA %**	**Compound Name**	**REF**	**CAS #**
1	12.326	99 %	S- pyridine 3-(1-methyl-2-pyrrolidinyl)	32085	000054-11-5
2	16.336	1 %	cyclohexylmethyl Isobutyl ester cyclohexylmethyl ester	88703 112139	1000309-21-3 1000309-21-6
Total	32 Min	100%	3 compounds	------	------

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Conclusion

In this study, we can conclude that the nicotine high purity rose 99% after converting the crude nicotine to its esters and the technique used in this study can be adopted to purify nicotine at least from Iraqi Tobacco.

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Conflict of Interest : None

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