Separation Identification and Determination of Nicotinic Acids and Nicotinamide in Cigarette Tobacco and Smoke (Part-I)

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(Received 4th March, 1997, revised 13th October, 1997)

Summary: Nicotinic acid and Nicotinamide were separated, identified and determined in cigarette tobacco and smoke by reverse phase HPLC with UV detection using water methanol (1:1) as mobile phase. Optimum solvent, volume of solvent, temperature and time were investigated for the extraction of nicotinic acid and nicotinamide from tobacco. All available brands of cigarettes tobacco and cigarette smoke were analyzed at optimum conditions and the results are presented and discussed.

Introduction

Environmental tobacco smoke (ETS) is the material released into the surrounding air by smoking tobacco products, Cigarettes, cigars and pipes all produce environmental tobacco smoke but cigarettes are the primary concern and nearly the sole focus of environmental tobacco smoke research, because of their wide spread use. Cigarettes derived environmental tobacco smoke results primarily from the smoke of the burning tip and that exhaled by smokers. It is generally agreed that the side stream smoke that released directly into the air from the burning tip is the major contributes to ETS. The smoke released from the burning tip of the cigarettes, cigars and pipes, not only affects the health of smoker but also affects the health of nonsmoker.

Smoke consists of about 400 to more than 1200 components. These components are determined by different methods. The well known alkaloid in tobacco Nicotine is determined by different methods like spectrophotometric [1-4], Gas chromatography [5-7], liquid chromatography [8] and pulse polarography [9].

In the present work HPLC technique has been exploited for determination of nicotinic acid and nicotinamide in cigarette tobacco and smoke.

Results and Discussion

Optimum solvent was investigated for maximum extraction of nicotinic acid and nicotinamide from cigarettes tobacco and for absorption of these components from cigarette smoke. The results are given in Table-1. Methanol was found to be an efficient solvent among the solvents investigated. However when coupled with stirring, heating, larger solvents volume, nicotinic acid and nicotinamide was completely extracted with water as a solvent. Therefore water was chosen as a solvent for further investigations.

Volume of water was optimized for the extraction from cigarette tobacco and cigarette smoke to find out the optimum w/v ratio of efficient extraction and v/v ratio for maximum dissolution of the component from the smoke. The results are given in Table-2. For extraction from tobacco, 50 ml was found to be the optimum volume for extraction as

Table-1: Solvent optimization for the extraction of nicotinic acid and nicotinamide from cigarette tabacco and cigarette smoke

Solvent	Tobacco (%)		Smoke (µg/cig.)	
	Nicotinic Acid	Nicotin-amide	Nicotinic Acid	Nicotin-amide
Water	0.45	0.625	112	440
Methanol	0.706	0.834	134	428
Ethanol	0.08	0.126	100	308

Table-2: Volume optimization for the extraction of Nicotinic acid and nicotinamide from cigarrette tobacco and cigarette smoke

Volume of solvent (ml)	Tobacco (%)		Smoke (µg/cig.)	
	Nicotinic Acid	Nicotin-amide	Nicotinic Acid	Nicotin-amide
20	0.420	0.599	260	146
30	0.443	0.617	330	200
40	0.622	0.625	214	270
50	0.800	0.657	325	270
60	0.800	0.687	321	275

further increase in volume did not result in increase in percent extraction of nicotinic acid and nicotinamide. In case of smoke, increase in the concentration of nicotinic acid and nicotinamide was observed with increase in volume of the absorption flask. This could be due to the increase in equilibration of the smoke in larger volume in absorption tube.

Optimum condition of equilibration time and temperature for the extraction of nicotinic acid and nicotinamide from cigarette tobacco was investigated. The results are given in Table-3-4. A period of 60 minutes was found to be the optimum equilibration time for maximum extraction while 80°C was found to be optimum temperature for efficient extraction.

Nicotinic acid and nicotinamide was determined in different brands of cigarettes tobacco at optimum conditions using reverse phase HPLC. Almost 100% efficiency of extraction was ensured using multiple extraction until there was no further extraction of these constituents. The results are given in Table-5. As can be seen from the table that variable amount of nicotinic acid and nicotinamide is present in the tobacco of different brands of cigarettes, the value being highest in Ranger and minimum in Chief. A good correlation between the concentration of these two species exist in different brands of cigarettes. The value of nicotinic acid and nicotinamide increase and decrease together equally in all samples investigated.

Table-3: Investigation of the effect of time on the extraction efficiency of nicotinic acid and nicotinamide from cigarette tobacco.

Equilibration time (minutes)	Percent Concentration	
	Nicotinic Acid	Nicotinamide
30	0.336	0.493
40	0.810	0.700
50	0.975	0.640
60	1.297	1.200

Conditions:
Weight of sample = 0.2 g
Total volume of solvent for extraction = 60 ml
Flow rate of mobile phase = 0.5 ml/min
Detector = UV
Column = Pecosphere C-18

Table-4: The effect of temperature on extraction efficiency of nicotinic acid and nicotinamide from cigarette tobacco.

Temperature (°C)	Percent Concentration	
	Nicotinic Acid	Nicotinamide
70	0.531	0.671
80	0.850	0.725
90	0.825	0.650
100	0.525	0.505

Conditions:
Time for extraction = 60 minutes
Weight of sample = 0.2 g
Total volume of solvent for extraction = 60 ml
Flow rate of mobile phase = 0.5 ml/min
Detector = UV
Column = Pecosphere C-18

Table-5: Determination of nicotinic acid and nicotinamide in different brands of cigarettes tobacco.

Name of Brands	Percent Nicotinic acid	Percent Nicotinamide
Ranger	0.835 ± 0.001	0.938 ± 0.01
Gold Leaf	0.517 ± 0.016	0.550 ± 0.06
Red and White	0.383 ± 0.006	0.429 ± 0.01
K-2	0.460 ± 0.054	0.557 ± 0.02
Cash	0.411 ± 0.010	0.507 ± 0.001
Tander	0.453 ± 0.014	0.501 ± 0.03
Chief	0.370 ± 0.007	0.381 ± 0.02

Nicotinic acid and nicotinamide were determined in the smoke of all brands of cigarette at

optimum conditions. The results are given in Table-6. Nicotinic acid and nicotinamide contents were variable in different brands of cigarette smoke. Maximum amount of nicotinic acid was observed in Red & White and minimum in Tander. Similarly maximum concentration of nicotinamide was observed in Red & White and minimum in Tander.

Table-6: Nicotinic acid and nicotinamide contents in the smoke of selected brands of cigarettes

Name of Brands	Nicotinic acid (µg/Cig.)	Nicotinamide (µg/Cig.)
Gold Leaf	175	80
Gold Cup	168	82
Ranger	288	85
Tander	105	75
Chief	135	80
K-2	130	156
Red & White	325	200
Cash	110	95

Experimental

Instruments

Bacharch, Coleman TriDet HPLC with Perkin Elemer 100 series pump and recorder was used during this work.

Reagents

Nicotinic acid, nicotinamide and methanol of Analar Reagent Grade were used throughout the analysis without further purification.

Solutions

1. *Stock solution of nicotinic acid:*

   1000 ppm solution was prepared by dissolving 0.1 g of nicotinic acid in distilled water and diluted upto 100 ml.

2. *Stock solution of nicotinamide:*

   1000 ppm solution of nicotinamide was prepared by dissolving 0.1 g of nicotinamide in distilled water and diluted upto 100 ml.

3. *Mobile Phase:*

   Water/Methanol was used as a mobile phase in 1:1 (v/v) ratio.

Procedure

Cigarette smoke from the selected brands of cigarettes was absorbed in 50 ml of distilled water using artificial smoker as shown in figure 1. All the samples were filtered using sintered glass crucible and water pump.

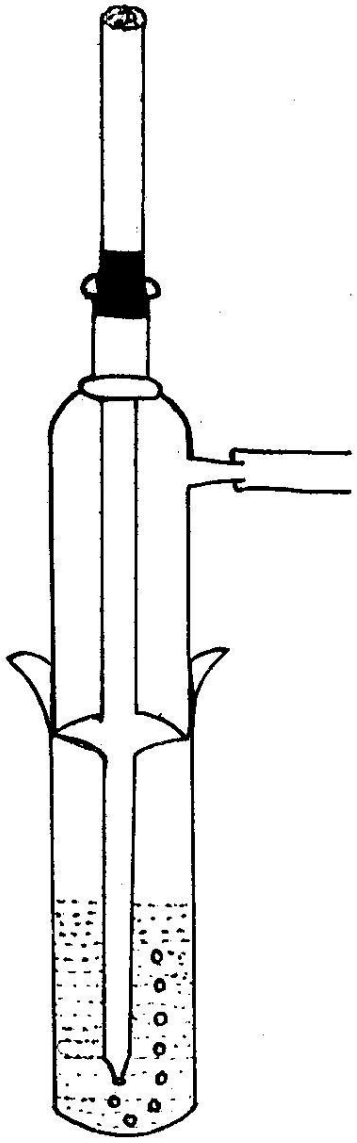

Fig. 1: Artificial Smoker

For extraction from tobacco, 20 ml of solvent was taken in a beaker and added 0.2 gram of tobacco of the selected brand of cigarettes to it. The resulting samples were swirled gently for about 60 minutes and then filtered using sintered glass crucible and water pump.

Samples of the tobacco extract and smoke were injected into the HPLC using reverse phase

chromatography with water methanol (1:1 v/v) as mobile phase and uv detector.

Conclusion

Optimum condition for extraction and separation of nicotinic acid and nicotinamide from cigarette tobacco and optimum volume for absorption of these components from cigarette smoke were investigated. It was observed that 60 ml solvent, 80°C temperature and 60 minutes heating time were the optimum conditions for extraction of nicotinic acid and nicotinamide from cigarette tobacco while 50 ml volume was found to be the optimum volume for absorption of these components from cigarette smoke. Nicotinic acid and nicotinamide both were found to be maximum in Ranger and minimum in Chief showing a good correlation among themselves. In case of cigarette smoke nicotinic acid and nicotinamide were found to be maximum in Red & White and minimum in Tander.

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