Optimum Condition Approach To Quantitative Analysis Of Nicotine Using HPLC

Jaruwan Chutrtong

Abstract: In order to find the optimum conditions for the nicotine analysis using high-performance liquid chromatographic (HPLC) technique, validation of mobile phase and flow rate were tested. Chromatographic conditions used for the experiment were zorbax SB-C 18 Column (150 × 4.6 mm, particle size 5 µm and 100 Å) with the maintained temperature at 35 ◦C. The UV detection was achieved at 260 nm with a bandwidth of 4 nm. and the injection volume for the analysis of all samples was 20 µL. The result shows that the optimum mobile phase for nicotine analysis was buffer NaH2PO4+ H3PO4 +Triethylamine: Acetonitrile: Methanol = 95:2:3 with a flow rate of 0.5 mL/min. It made the appropriate elution time and has the good chromatogram which is good for the nicotine quantity analysis.

Index Terms: High Performance Liquid Chromatography, Nicotine, Optimum condition, Quantitative.

1 INTRODUCTION

Nicotine is the primary alkaloid found in tobacco (Nicotiana tobacum) [1]. It can penetrate easily through human and animal skin and evaporate easily at room temperature. Nicotine in the form of free bases can be burned and evaporated at 95 degrees Celsius [2]. Nicotine acts through a nicotinic receptors, result in increased secretion of adrenaline [3]. High dose of nicotine can cause the suspension of acetylcholine receptors and nicotine poisoning. Nicotine is considered a very toxic substance [4]. For the toxicity, its LD50 is 50 mg/kg in medium rats and 3 mg/kg in small rats. In human, the estimated lethal adult oral dose of nicotine is between 40 and 60 mg, or approximately 0.6 to 0.9 mg/kg [5]. Although nicotine is a dangerous substance, the consumption of nicotine is still widespread and legal. Usually, people take nicotine by cigarette smoking. Extract or synthetic nicotine are used as components in chewing gum, skin patch or nasal spray use in cigarette addicts to reduce smoking, which is more harmful to health. In addition, nicotine is also an important component of electronic cigarettes, which are growing in popularity exponentially. The production of such products is necessary to have accurate quality control especially the quantity and purity of nicotine, for effective use and safety. Several high-performance liquid chromatographic (HPLC) methods are available for the quantification of nicotine [6]. From previous research, nicotine quantity analyze by using the high performance liquid chromatography technique, sometime, give unbalanced and broken peak which make the inexact analysis results interpretation [7][8]. Moreover, the preparation of the mobile phase is complicated. The goal of this research is develop suitable condition for the nicotine analysis in order to get balanced peak. The hassle-free and saving mobile phase preparation is also the goal. Standards of the alkaloids nicotine are prepared and quantified using high performance liquid chromatography. Various elution schemes are tested and adjusted for optimal analyze resolution.

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2 PROCEDURE

2.1 Materials

Nicotine Assay 95.58% were purchased from Millimed Chemical Company. The analytical reagent (AR) and HPLC grade chemical reagents were purchased from a retail outlet in Bangkok, Thailand. The water used in the formulation of buffers obtained by the process of reverse osmosis (0.22 µm Millipore).

2.2 Nicotine standard solution preparation

The 1000 µg/mL nicotine standard solution was diluted with HPLC grade methyl alcohol and water to concentrations of 100 µg/mL and filtrated with syringe filter pore size 0.45 µm.to make stock solutions.

2.3 Chromatographic Conditions Validation

Prepared 50 mL of 0.5M NaH_2PO_4, 0.5M Na_2HPO_4, 0.5M CH_3COONa, adjust the volume with distilled water and filter.

1. Adjusted the volume of the solvent in 1. with distilled water to 1000 mL
2. Dropped orthophosphoric acid (H_3PO_4), Glacial acetic acid, (CH_3COOH) ratios 1 drop per 500 mL of Buffer.
3. Prepared triethanolamine ($N(CH_2CH_2OH)_3$) by dissolved 1 drop of Triethanolamine in 9 drops of distilled water and filtered by syringe filter
4. Added 1 drop of triethanolamine solution to 100 mL of buffer.
5. Mix well.

2.4 Chromatographic Conditions Validation

An Agilent series 1100 HPLC (Agilent Technologies (Thailand) Co.,Ltd.) were used, comprising a quaternary pump, vacuum degasser, an autosampler, and a column compartment with thermostat and a photodiode array (PDA) detector, with data acquisition from Chemstation software(Agilent). Agilent zorbax SB-C 18 Column (150 × 4.6 mm, particle size 5 µm and 100 Å) was used, with the maintained temperature at 35 ◦C. Investigated mobile phases in different combinations and, different flow rates. The UV detection was achieved at 260 nm with a bandwidth of 4 nm. The mobile phases were filtered by using a 0.45 µm filter and degassed by sonication for 10 min before use. The injection volume for the analysis of all samples was 20 µL. Mobile Phase and Flow Rate were validated the as in table 1.

TABLE 1
UNITS FOR MAGENTIC PROPERTIES

	Separate system	*ratio*	*Flow Rate mL/min*
1	*Buffer A : Acetonitrile*	*95:5*	*1.0*
2	*Buffer A : Acetonitrile*	*95:5*	*0.5*
3	*Buffer A : Acetonitrile : Methanol*	*95:2:3*	*0.5*
4	*Buffer A : Acetonitrile : Methanol*	*90:2:8*	*0.5*
5	*Buffer NaH_2PO_4*	*100*	*1.0*
6	*Buffer B*	*100*	*1.0*
7	*Buffer B : Acetonitrile*	*95:5*	*1.0*
8	*Buffer C*	*100*	*1.0*
9	*Buffer C : Acetonitrile*	*95:5*	*1.0*
10	*Buffer C +Triethylamine*	*100*	*1.0*

Buffer A = NaH_2PO_4+ H_3PO_4 +Triethylamine
Buffer B = NaH_2PO_4+CH_3COOH
Buffer C = $C_2H_3Na0_2$+CH_3COOH

3 RESULTS AND DISCUSSION

The results of the research to find the optimum conditions for analyze 100 µg/mL standard nicotine solution using High Performance Liquid Chromatography, C18 columns and vary mobile phase condition as in Table 1. are shown in the following figure. Chromatogram in Figure 1 - 4 are the result which use buffer with NaH_2PO_4, H_3PO_4 and triethylamine as main component of mobile phase. Proportion of mobile phase of experiment in Figure 1 and 2 are the same but different flow rate. Mobile phase of Figure 3 and 4 are different from mobile phase of Figure 3 and 4. It has methanol. As well, mobile phase of experiment in Figure 3 and 4 are the same component but they have different proportion. They also use the same flow rate.

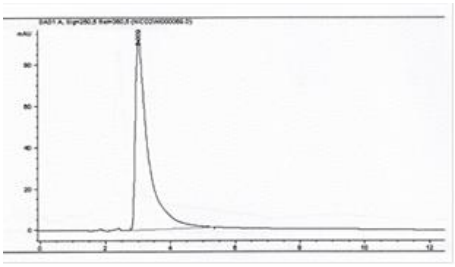

FIG. 1*. CHROMATOGRAM OF NICOTINE STANDARD SOLUTION AT 260 NM. MOBILE PHASE IS BUFFER NAH2PO4+ H3PO4 +TRIETHYLAMINE: ACETONITRILE = 95:5, FLOW RATE 1.0 ML/MIN.*

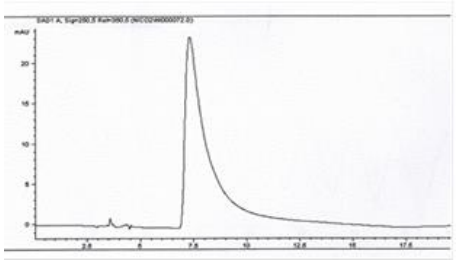

Fig. 2*. Chromatogram of nicotine standard solution at 260 nm. Mobile phase is buffer NaH2PO4+ H3PO4 +Triethylamine: Acetonitrile = 95:5, Flow Rate 0.5 mL/min*

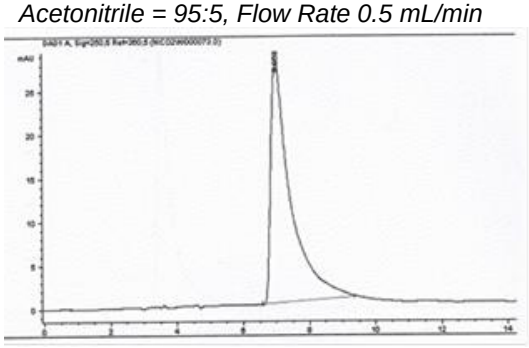

Fig. 3*. Chromatogram of nicotine standard solution at 260 nm. Mobile phase is buffer NaH2PO4 + H3PO4 +Triethylamine: Acetonitrile: Methanol = 95:2:3, Flow Rate 0.5 mL/min.*

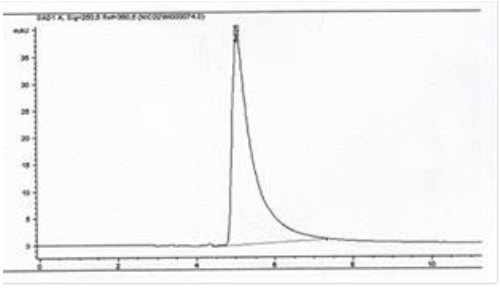

Fig. 4*. Chromatogram of nicotine standard solution at 260 nm. Mobile phase is buffer NaH2PO4+ H3PO4 +Triethylamine: Acetonitrile: Methanol = 90:2:8, Flow Rate 0.5 mL/min.*

Experiment in Figure 5 use only buffer NaH_2PO_4 as mobile phase and the flow rate is 1.0 mL/min.

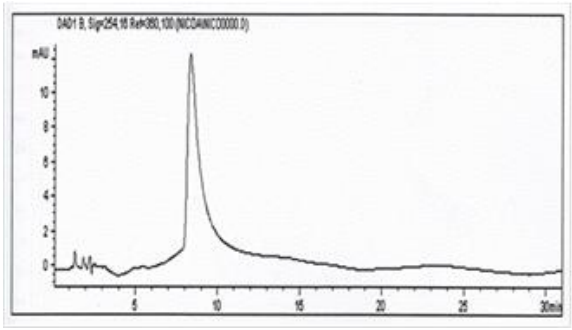

FIG. 5*. CHROMATOGRAM OF NICOTINE STANDARD SOLUTION AT 260 NM. MOBILE PHASE IS BUFFER NAH2PO4. FLOW RATE 1 ML/MIN*

From the chromatogram, condition 1-5, nicotine eluted for no more than 10 minutes but there were differences in duration. The substance eluted quite quickly in first and fifth condition. The characteristics of the peak of condition 1-5 are quite good. Another thing to concern is the base line. Good base line should be smooth. Base line of the fifth condition not smooth. For the experiments in conditions 6-10, the substance was retained in the column for more than 10 minutes. Get stuck long time in column of substance affect to the analysis time. Therefore, the analysis results of such conditions are not used

for comparison.

3 CONCLUSION

From the results shown with chromatography, it can be concluded that the suitable condition for nicotine analysis is the third condition, buffer NaH2PO4+ H3PO4 +Triethylamine : Acetonitrile : Methanol = 95:2:3 with flow rate 0.5 mL/min. Consider from the chromatogram, it is found that the elution time of nicotine this condition is appropriate. The substance was released at approximate 7 minute. This time is enough to separate nicotine from impurity. If use for samples analysis, it will get the correct result. In addition, it was found that the analytical signal had a good response, sharp peak and smooth base line. For other conditions, the first and fifth condition elute time quite quickly. The release of substances too quickly may cause it to overlap with impurity when analyze real samples. Base line of the second and fifth condition are not smooth. Comparing the peak sharp, the third is the best. This method can speed up the elute time by adjust the proportion of solvent if confident in the purity of samples.

4 ACKNOWLEDGMENT

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