

ANALYSIS OF NICOTINE AND COTININE IN TOBACCO INDUSTRY WORKERS

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Abstract. The present study aimed to follow up the levels of nicotine and its major metabolite, cotinine, in workers' urine of tobacco industry, using a reliable gas chromatographic method. The limit of detection for both nicotine and cotinine was 10 ng ml⁻¹. We consider that the urinary nicotine and cotinine levels might be served as biological monitoring index for estimation of the exposure level for the non-smoking workers in tobacco industry.

Key words: nicotine, cotinine, chromatography, occupational exposure

Rezumat. Acest studiu a avut drept scop determinarea, prin intermediul unei metode gaz cromatografice sigure, a nicotinei și principalului său metabolit, cotinina, din urina muncitorilor ce lucrează în industria tutunului. Limita de detecție a fost de 10 ng. ml⁻¹, atât pentru nicotină, cât și pentru cotinină. Considerăm că nivelurile urinare de nicotină și cotinină pot fi utilizate drept indice biologic de monitorizare, în estimarea gradului de expunere profesională pentru muncitorii nefumători din industria tutunului.

Cuvinte cheie: nicotină, cotinină, cromatografie, expunere profesională

INTRODUCTION

The occupational exposure to tobacco dust may be associated with the development of asthma, bronchitis, dermatitis and conjunctivitis [1,2,3].

Tobacco leaves contain approximately 2,500 chemical compounds, among which there are 14 alkaloids [4]. The most important alkaloid is nicotine; it is absorbed through the lungs, skin and by ingestion and then rapidly passes into blood.

Nicotine is metabolized primarily to cotinine; the half-life is 2-8 h for nicotine and 20 h for cotinine [5,6,7,8,9]. The urinary levels of unmodified

nicotine are small, but the amount of cotinine excreted in urine is high.

Therefore, in this work, a gas chromatographic method has been developed for the simultaneous measurement of nicotine and cotinine in human urine. The levels of nicotine and cotinine were correlated with airborne tobacco dust concentration in the working areas.

MATERIALS AND METHODS

Subjects. Three groups of subjects were studied: one group of 15 non-smoker workers (having an average duration of service of 13.3 years), the

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second group of 5 smoker workers (having an average duration of service of 9.6 years) and a control group comprising 10 cigarette smokers with no occupational exposure to tobacco dust.

Urine analysis procedure. First morning urine-spot in the studied groups was collected.

To 20 ml urine were added 3.2 g of NaOH. 7 ml chloroform were then added and mixed for 15 minutes. The layers were separated by gentle centrifugation for one minute. The chloroform extract was then transferred to a tapered centrifuge tube and evaporated down to approximately 0.5 ml at 37⁰ C; then 0.5 µl of concentrated extract were injected into gas chromatograph.

Chromatographic conditions. A Carlo Erba model Fractovap 2450 gas chromatograph equipped with a flame ionization detector was used.

A glass column (0.8m x3 mm i.d.) was used, with a stationary phase of Apiezon L + 2% KOH on Volaspher A1 120-140 mesh (Pye Unicam). Hydrogen was used as the carrier gas with a flow-rate of 30 ml/minute. The temperature of the injection port and detector was of 275⁰ C.

Gas chromatographic analysis was performed in isothermal conditions at 180⁰ C.

The external standard method was used as calculus method. We used standards of nicotine [(+)-di-p-toluytartrate] and (-)cotinine [(1-methyl-5-[3-pyridyl]-2-pyrrolidinona)] (Sigma,USA) with 0; 30; 50; 100; 220; 660 ng .ml⁻¹ dissolved in CHCl₃.

In order to calculate nicotine:creatinine and cotinine:creatinine ratios, the creatinine concentration of each urine sample was measured.

Total amount of dust in the working areas was measured by gravimetric method.

RESULTS AND DISCUSSION

A typical chromatogram of an extract from human urine showed retention times of 1.5 min. for nicotine and 7 min. for cotinine.

Calibration curve showed a linear response over the range of 10-400 ng.ml⁻¹ for nicotine and 100-700 ng.ml⁻¹ for cotinine.

The limit of detection for both nicotine and cotinine appears to be 10 ng.ml⁻¹.

The reproducibility over the concentration ranged between 1.0 to 600.0 ng.ml⁻¹ for both nicotine and cotinine in human urine and is shown in table 1. The average coefficient of variation over the nicotine and cotinine range was 2.3 and 4.9 respectively.

This method has been applied to the determination of nicotine and cotinine in tobacco industry workers and the results are presented in table 2. Table 3 shows the statistical significance of the nicotine and cotinine values difference in the three studied groups. Regression analysis does not show correlation between urinary nicotine and cotinine levels and length of service of exposed workers.

Table 1. Reproducibility of results of five determinations at five nicotine and cotinine concentrations in human urine

Nicotine				Cotinine			
Added ng.ml ⁻¹	Found ng.ml ⁻¹	S.D.	CV%*	Added ng.ml ⁻¹	Found ng.ml ⁻¹	S.D.**	CV%
10.0	9.7	0.60	6.1	10.0	10.9	0.66	6.0
100.0	103.5	0.92	0.8	100.0	98.4	2.30	2.3
200.0	197.2	1.58	0.8	200.0	204.0	7.95	3.9
400.0	397.0	4.76	1.2	400.0	404.1	6.86	1.7
600.0	605.2	13.9	2.3	600.0	594.0	11.28	1.9

*Coefficient of variation based on five replicate analysis

**Standard deviation

Table 2 Urinary concentrations of nicotine and cotinine in nonexposed and exposed subjects in tobacco dust

Group	No.subjects	Nicotine Mean±S.D. µg/100mg creatinine	Cotinine Mean±S.D. µg/100mg creatinine
Controls	10	58.05±18.43	55.56±22.05
Smoker workers	5	105.55±43.03	74.67±14.17
Non-smoker workers	15	47.69±34.94	27.15±24.57

Table 3 Statistical significance of the nicotine and cotinine values difference in the studied groups

Compared groups	Statistical significance of the values difference*	
	Nicotine	Cotinine
Controls-smoker workers	p>0.05	p>0.05
Controls-nonsmoker workers	p>0.05	p<0.05
Smoker workers-nonsmoker workers	p<0.05	p<0.05

*The Student test was used

The concentration of nicotine and cotinine excreted in urine by non-smoker exposed workers is of comparable range with that found in cigarette smokers (control). Therefore we can state that urinary nicotine level might be used as a biological index for estimation of the occupational

exposure in tobacco industry for the non-smoker workers. This is in agreement with the results of other researchers [10].

A positive correlation was found between urinary cotinine level and tobacco dust concentration in the

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working areas ($r=+0.60$, $p>0.05$). This fact indicates that urinary cotinine level might be used as a biological index for occupational exposure to tobacco in non-smoker workers.

CONCLUSIONS

- ◆ Taking into account the results obtained in this work we can conclude that this method is reliable and may be used for the simultaneous determination of nicotine and cotinine in human urine.
- ◆ The urinary nicotine and cotinine levels may be used as biological monitoring indicators in tobacco industry non-smoker workers.

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