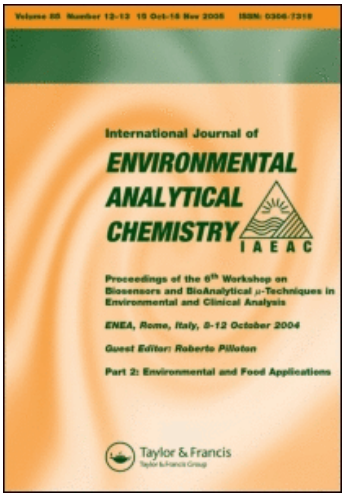

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The aim of the present study was to develop a new analytical method of chromatographic determination of two important markers of ETS exposure: nicotine and 3-vinylpyridine (3-ethenylpyridine, 3-EP) in mainstream (MS) and sidestream (SS) smoke of one single cigarette and in indoor air using direct solid phase extraction combined with gas chromatography. The method can be utilised for both nicotine and 3-EP determination in SS and MS of one single cigarette as well as it allows for a precise determination of compound distribution in indoor air. The application of the same analytical method for both kinds of samples allows anticipating indoor air distribution of both analysed compounds in a very precise way. The precision of the method (calculated as a relative standard deviation) was 9.78% for nicotine and 2.67% for 3-EP; whereas the accuracy (evaluated by a recovery study conducted at three different levels) was 70.1 and 87.3%, respectively. The limit of detection was 0.06 μg per cigarette for both nicotine and 3-EP. The method was evaluated by determining the compounds of interest in two commercially available brands of cigarettes as well as in the reference cigarettes 3R4F and also in indoor air polluted with tobacco smoke. Determined levels of compounds of interest in MS varied from 586 to 772 (nicotine) μg per cigarette and from 3.5 to 10.7 (3-EP) μg per cigarette. In SS smoke the level varied from 14,370 to 22,590 (nicotine) μg per cigarette and from 185 to 550 (3-EP) μg per cigarette, whereas levels in indoor air polluted with tobacco smoke varied from 50.1 to 157.3 (nicotine) $\mu g\,m^{-3}$and from 7.7 to 20.8 (3-EP) $\mu g\,m^{-3}$.

Keywords: nicotine; 3-vinylpyridine; indoor air; gas chromatography; SPE; tobacco smoke; cigarettes

1. Introduction

One of the most significant sources of environmental exposure to many toxic compounds is environmental tobacco smoke (ETS). ETS is a complex mixture that is produced between puffs primarily by the release of smoke from the burning cone (i.e. sidestream smoke, SS) and the smoke exhaled by the active smoker. Other components of ETS

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include the mainstream smoke emitted from the mouthpiece of cigarettes and the vapour compounds that diffuse through the cigarette wrapper. Mainstream smoke (MS) is a fraction of the tobacco smoke that is actively inhaled by active smokers.

Exposure as a result of staying in a room polluted with ETS is referred to as passive smoking. Statistical data indicate that such exposure affects the vast majority of the world's population. Passive smokers exposed to ETS are at increased lung cancer risk (between 20% and 30%), and at 25% increased risk of heart disease [1]. Epidemiological research has proved a statistically significant connection between passive smoking and the occurrence of many disorders [1,2]. Because of low exposure to tobacco-derived toxic substances present in the indoor air polluted with tobacco smoke, such connections (by comparison with active smokers) are revealed only in studies involving very large populations [3]. Moreover, by contrast with active smokers' exposure, the exposure to ETS is highly varied. It depends on many factors often difficult to specify. Research projects which quite objectively diversify passive smokers' exposure consist of: (1) specifying doses which are taken by a passive smoker while inhaling indoor air polluted with tobacco smoke, or (2) specifying the concentrations of ETS components which a passive smoker is exposed to. Due to a wide range of ETS toxic components, the determination of only selected substances is carried out. These substances are called markers of exposure and thus approximate ETS as a whole. By determining markers as well as biomarkers of exposure it might be possible to assess bioaccumulation (i.e. disposition in a body) of various toxic compounds from tobacco smoke.

The aim of the study was to develop a method of chromatographic determination of two important markers of ETS exposure, namely nicotine and 3-vinylpyridine (3-ethenylpyridine, 3-EP) in MS and SS of a single cigarette and in indoor air, using solid phase extraction (SPE). The method should determine both markers in single cigarette smoke so as to control the diversification of nicotine and 3-EP levels in tobacco smoke generated from cigarettes of the same brand.

2. Experimental

2.1 *Reagents*

The *standard solution* of nicotine and 4-vinylpyridine (4-ethenylpyridine, 4-EP) was obtained by dissolving calculated masses of those substances (extra pure chromatographic standards of nicotine (Sigma Aldrich, USA) and 4-EP (Alfa Aesar GmbH&CoKG, Germany) in redistilled water. Nicotine and 4-EP concentrations were 20.17 and $0.59\,\mathrm{mg\,mL^{-1}}$, respectively. On account of 3-EP instability and its unavailability, 4-EP is commonly used as a substitute of this compound, especially in standardized ISO method [5]. 4-EP has been proven to have an identical retention time as 3-EP and give the same peak values and detector response [4,5]. *Calibration solutions* of nicotine and 4-EP were obtained by dissolving calculated masses of those substances in methanol and successive diluting. Concentrations of the calibration solutions varied from 1×10^{-1} to 1×10^{4} and from 5×10^{-1} to $5 \times 10^{3}\,\mu\mathrm{g\,mL^{-1}}$ for nicotine and 4-EP, respectively. The following sorbents were examined: Chromosorb G, Chromosorb W, Chromosorb 102, Chromosorb 101, Chromosorb T, Porapak N, Porapak S, Porapak P, Porapak T, Porapak QS, Porapak Q, Porapak R (Supelco, USA). Physiochemical properties of the sorbents are presented in Table 1. Just before use, all sorbents were activated and conditioned during a 2-h period at the temperature of 180°C. The SPE columns used in the experiments were packed with

Table 1. Physiochemical characteristics of examined sorbents.

Sorbent name	Chemical characteristics	Mesh
Chromosorb G	Diatomite	80/100
Chromosorb W	Diatomite	80/100
Chromosorb T	Tetrafluoethylene polymer	60/80
Chromosorb 101	Styrene/divinylbenzene copolymer	80/100
Chromosorb 102	Styrene/divinylbenzene copolymer	80/100
Porapak QS	Styrene/divinylbenzene copolymer	80/100
Porapak R	Styrene/divinylbenzene copolymer	80/100
Porapak N	Styrene/divinylbenzene copolymer	80/100
Porapak S	Styrene/divinylbenzene copolymer	80/100
Porapak P	Styrene/divinylbenzene copolymer	80/100
Porapak T	Styrene/divinylbenzene copolymer	80/100
Porapak Q	Styrene/divinylbenzene copolymer	100/120

each sorbent of various mass, and then the sorbent bed was washed with methanol and dried in an extra pure nitrogen stream. The following eluents have been used: methanol, acetone, ethyl acetate, cyclohexane, 1,2-dichloroethane and carbon tetrachloride (all extra pure grade, POCh, Poland).

2.2 *Gas chromatographic determination of nicotine and 3-EP*

All experiments described in this article were performed using: gas chromatograph Varian CP-3800 with capillary column CP-Sil 8CB 25 m × 0.25 mm (1.2 μm) and FID detector (Varian Corporation, USA); vacuum manifold for SPE (J.T. Baker, USA); flow meters (Rotametr, Poland).

Nicotine and 3-EP were identified on the basis of their retention times, which were established experimentally by examining the standard solution. The quantitative determination of nicotine and 3-EP in samples was conducted by comparing unknown sample peak values with peak values in the standard solution, after proving the rectilinearity of relationship between the peak value and the concentration of examined compounds. Gas chromatography parameters were selected in such a way as to reach adequate separation of analysed compounds while maintaining the shortest possible time of analysis. Helium was used as carrier gas, and gas flows were: helium ($3\,mL\,min^{-1}$), hydrogen ($10\,mL\,min^{-1}$) and air ($300\,mL\,min^{-1}$). The temperature of the oven was programmed from 50 to 275°C. The full analysis took 22.5 min. All analyses were conducted in the same conditions. The retention time for 3-EP and nicotine amounted to 7.01 and 12.89 min, respectively. Preliminary data with GC–MS confirmed the identity of analysed peaks. Moreover, identical retention time and flame ionization detector response for 3-EP and 4-EP were confirmed by conducting experiments with various columns and temperature programs. Within-day and day-to-day variation in retention times of both compounds were less than 5%.

2.3 *Optimisation of SPE method*

In order to obtain an optimum simultaneous extraction of nicotine and 3-EP from MS, SS and ETS, using the SPE, a series of experiments were performed to select both the best sorbent and most efficient eluent, as well as their optimum quantities.

The following items were subject to optimisation:

- selection of sorbent which retains nicotine and 3-EP from MS, SS and ETS, and eluent which guarantees maximum recovery of the adsorbed compounds (Step 1),
- selection of optimum sorbent mass (Step 2) and optimum eluent volume (Step 3).

In Step 1 of the optimisation process we used aqueous standard solutions of nicotine and 4-EP. Such an approach enabled rapid screening for sorbent–eluent pair of the highest sorption–desorption efficiency. Whereas during Steps 2 and 3 nicotine and 4-EP vapours were generated.

2.3.1 *Selection of the optimal sorbent–eluent combination (Step 1)*

In Step 1, 11 types of sorbents and 6 types of eluents were examined. Hence, 66 SPE sorbent–eluent combinations were screened. SPE columns were packed with 0.5 g of sorbent. Then 1 mL of standard solution containing both examined compounds (amounts corresponding to average levels in SS tobacco smoke generated from one cigarette) was passed through the columns at constant flow, followed by washing the columns with 2 mL of examined eluents. All 66 extracts were analysed by means of the GC–FID. On the basis of obtained results, the total efficiency of both extraction and re-extraction processes of nicotine and 4-EP were calculated for each examined combination.

2.3.2 *Selection of the optimal sorbent mass (Step 2)*

After the best sorbent–eluent combination was found in Step 1, a series of experiments were performed during which varied masses of the selected sorbent were examined (0.1; 0.25; 0.5; 1.0; 2.0 g). The extraction procedure was different than described in Step 1. Instead of aqueous solutions, vapours of nicotine and 4-EP were generated in a vacuum flask by gentle heating of their standard solution with an electric heater. Generated vapours were transferred under pressure directly to a SPE tube with the stream of pure nitrogen. The break through of sampling system was tested using vapours generated from calibration solutions of increasing concentration of nicotine and 4-EP.

2.3.3 *Selection of the optimal eluent volume (Step 3)*

After an optimum sorbent mass was determined in Step 2, a series of experiments were performed during which varied volumes of the selected solvent were examined (1, 2, 3, 4 and 5 mL). The extraction procedure was the same as described above in Step 2, though by using the optimal sorbent mass.

2.4 *Determination of nicotine and 3-EP in tobacco smoke and indoor air*

Two commercially available cigarette brands and 3R4F reference cigarettes (University of Kentucky, USA) were applied for the method development and validation. Before use, the cigarettes were conditioned for 48 h in relative humidity of 60%, according to ISO standards [6]. MS and SS from a single cigarette were generated using a standard cigarette smoking machine described in detail in previous papers [7,8] (Figure 1), which meets all ISO requirements [6,9] (duration of a puff: 2 s, interval between puffs: 60 s, puff volume: 35 cm^3, linear velocity of SS removing from cigarette combustion chamber: 2 $cm\,s^{-1}$, butt

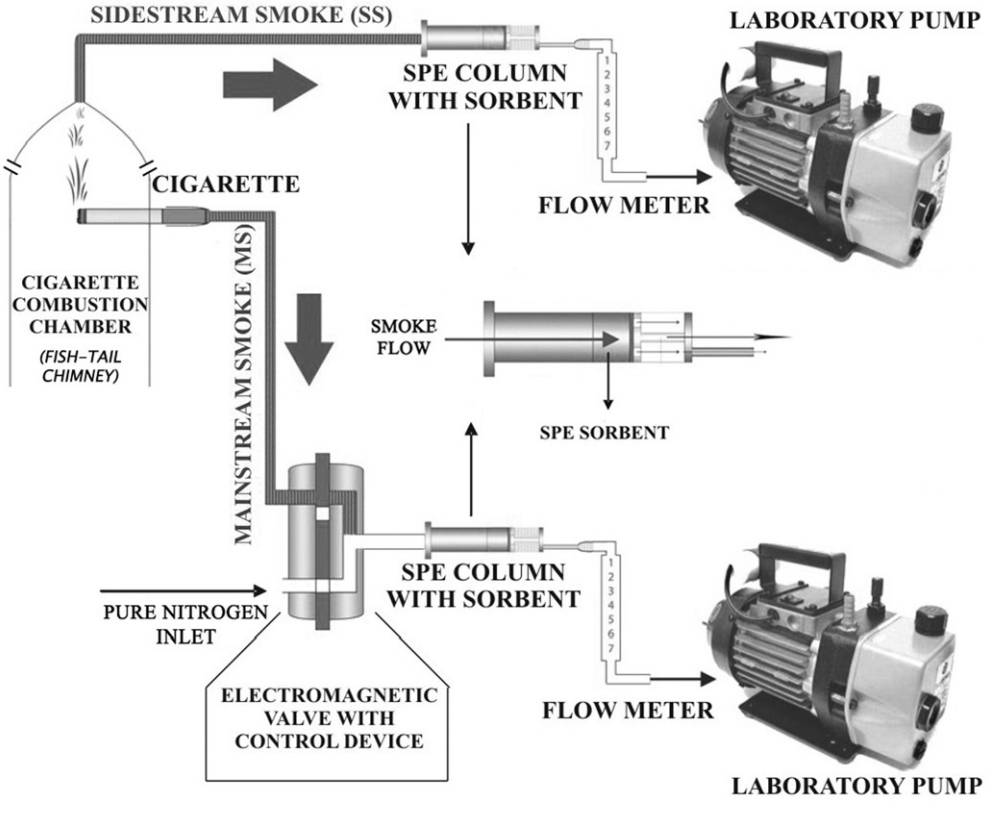

Figure 1. Cigarette smoking machine – scheme of MS and SS generation and absorption system.

length: 3 mm over mouthpiece). Generated MS and SS passed through SPE columns and as a result nicotine and 3-EP were retained on the sorbent. Afterwards, the compounds of interest were eluted from SPE columns and then the gas chromatographic determination was performed.

Nicotine and 3-EP were extracted from indoor air polluted with ETS. The sampling kit consisted of: SPE column packed with selected sorbent (column diameter: 15 mm), flow meter and vacuum pump. A volume of 1 m^3 was aspired with a stable flow of 0.33 $m^3\,h^{-1}$. The compounds of interest were determined in laboratory (ETS generated in controlled way) as well as in real-life conditions (apartments of active smokers and pub).

2.5 *Method validation*

The method was validated in terms of linearity, precision, recovery and accuracy. The limit of detection (LOD) and limit of quantification (LOQ) were also determined. In order to define the intra-day and inter-day precision of nicotine and 3-EP determination, a whole analytical procedure was performed four times over a day during a 1-week period using vapours generated from 10 μL of pure chromatographic standards of nicotine and 4-EP. In order to define the recovery of nicotine and 3-EP in the optimised chromatographic method, vapours generated from pure chromatographic standards of nicotine and 4-EP

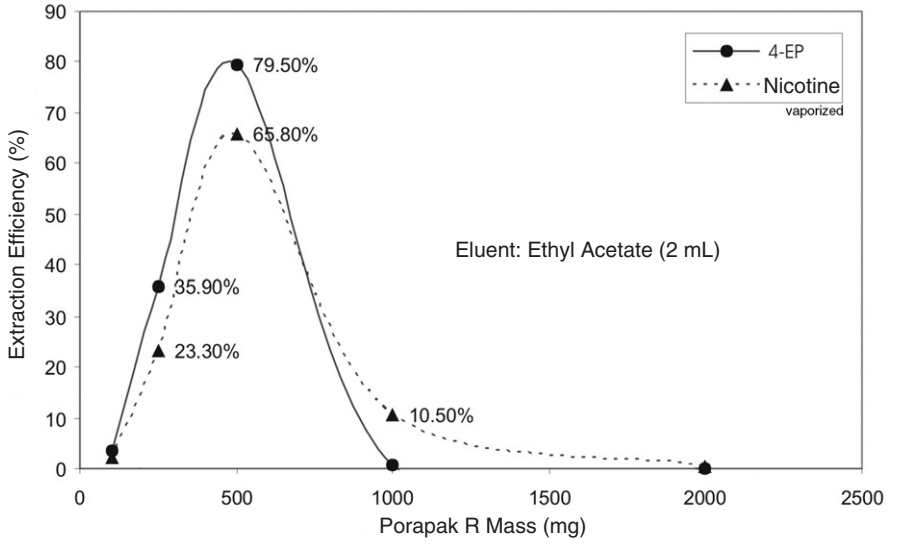

Figure 2. The results of sorbent mass optimisation (Step 2).

(each of 100 μL) were deposited onto a SPE column. The column was then washed with the selected solvent. In the obtained eluent, nicotine and 4-EP concentration was determined by means of the GC method. The accuracy of the method was assessed using 9 determinations over three concentration levels covering the specified range (corresponding to 1–300 $\mu g\,m^{-3}$ during 3-h sampling period). Generated vapours of the compounds of interest were used. The LOD was expressed as 3 : 1 and LOQ as 6 : 1 signal-to-noise ratio. All described procedures were also conducted for blank samples.

3. Results

3.1 *Results of the method development*

Results from Step 1 experiments established that the optimum sorbent was Porapak R and optimum eluent was ethyl acetate. The results of the sorbent mass optimisation (Step 2) are presented in Figure 2. The results of solvent volume optimisation (Step 3) are presented in Figure 3. The final results of SPE evaluation are as follows:

- sorbent: Porapak R (80–100 mesh) of 0.5 g
- eluent: ethyl acetate of 3 mL

In the conditions described above the best efficiency of extraction of nicotine and 3-EP (87 and 92%, respectively) was achieved. Assessed sorbent capacity was about 40 mg of nicotine and 10 mg of 3-EP.

3.2 *Results of nicotine and 3-EP determination in tobacco smoke and indoor air*

The worked out method was verified by quantification of both markers in two commercially available cigarette brands and indoor air polluted with ETS. The results

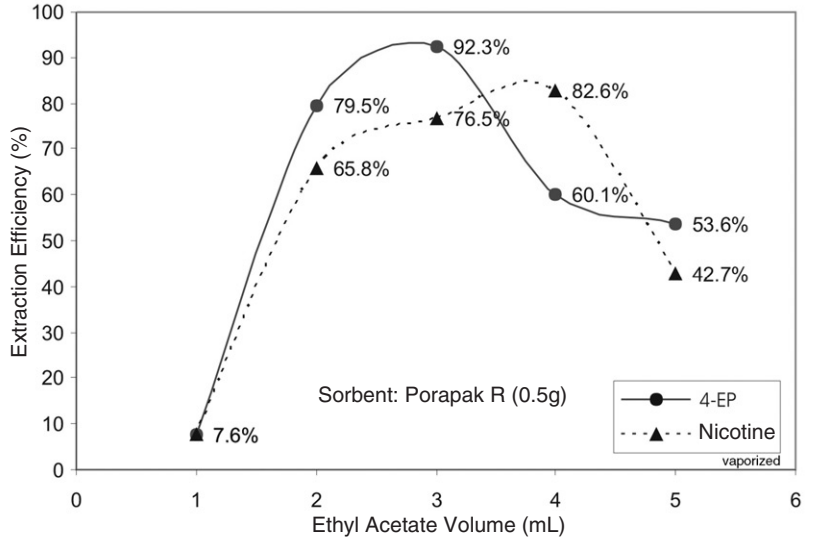

Figure 3. The results of solvent volume optimisation (Step 3).

are presented in Tables 2 and 3. An exemplary chromatogram of tobacco smoke is presented in Figure 4.

3.3 *Results of method validation*

The method was validated as described in Section 2.5. The validation of the worked out method of simultaneous determination of nicotine and 3-EP in MS and SS indicates that the method meets the criteria of determination in quantitative analytical chemistry concerning analysis of traces and pollution (Table 4). Calibration curves were linear over the range tested (correlation coefficient $r = 0.9969$ for nicotine and 0.9971 for 3-EP). The recoveries for nicotine and 3-EP were 91.3 and 95.4% for nicotine and 3-EP, respectively. Precision was calculated as a relative standard deviation (RSD) of the results based on the analyses of standardised vapours. The intra-day and inter-day RSDs were found to be 7.2 and 9.8% for nicotine and 1.7 and 2.7% for 3-EP, respectively. The accuracy of the method was 70.1% for nicotine and 87.3% for 3-EP (RDSs were less than 1%). Limit of detection was 0.06 μg per cigarette for nicotine and 3-EP. Limits of quantification were 0.18 and 0.19 μg per cigarette, respectively. For the determination of compounds of interest in indoor air limit of detection was 0.06 $\mu g\,m^{-3}$ for nicotine and 3-EP, and limits of quantification were 0.18 and 0.19 $\mu g\,m^{-3}$, respectively. In blank samples nicotine and 3-EP were not detected, so they did not influence both calibrations and measurements.

4. Discussion

The newly developed method meets all the requirements for modern analytical techniques, particularly in regards simplicity and universality. The method can be utilised for both nicotine and 3-EP determination in SS and MS of one single cigarette as well as in indoor air.

Table 2. Nicotine and 3-EP levels in MS and SS of examined cigarette brands.

	MS (μg/cigarette)			SS (μg/cigarette)			
Cigarette brand	One cigarette	Average level	SD	One cigarette	Average level	SD	MS/SS Ratio
Nicotine in cigarette smoke							
Brand A	605	666	53	14,370	14,750	801	1:22
Full Flavour	694			15,670			
	700			14,210			
Brand B	586	628	48	22,100	21,345	1749	1:31
Low tar	617			22,590			
	681			19,345			
3R4F	692	735	40	15,765	15,224	498	1:21
Reference	741			15,123			
	772			14,786			
3-*EP in cigarette smoke*							
Brand A	9.2	9.5	1.2	529	531	14	1:56
Full Flavour	10.7			518			
	8.5			545			
Brand B	11.6	10.9	1.1	550	499	45	1:46
Low tar	9.6			465			
	11.5			482			
3R4F	3.5	4.1	0.7	185	219	35	1:53
Reference	3.9			217			
	4.8			254			

Note: MS: Mainstream smoke; SS Sidestream smoke.

Table 3. Nicotine and 3-EP levels in indoor air polluted with tobacco smoke.

Indoor environment	Room capacity (m^3)	Number of smokers present in the room	Concentration in indoor air ($\mu g\,m^{-3}$)
Nicotine in indoor air			
Laboratory	70	Smoking machine	50.1
Apartment	15	2	157.3
Pub	180	19	155.1
3-EP in indoor air			
Laboratory	70	Smoking machine	11.8
Apartment	15	2	20.8
Pub	180	19	7.7

In our future project we are going to develop a mathematical model for forecasting indoor air concentrations of smoke constituents based on their generation rates from a single cigarette. By using the method described in this article, it will be possible to minimise potential errors that might influence the precision of the mathematical model. The authors did not find any described method, which would permit simultaneous determination of nicotine and 3-EP in two different sample types: tobacco smoke and indoor air (various

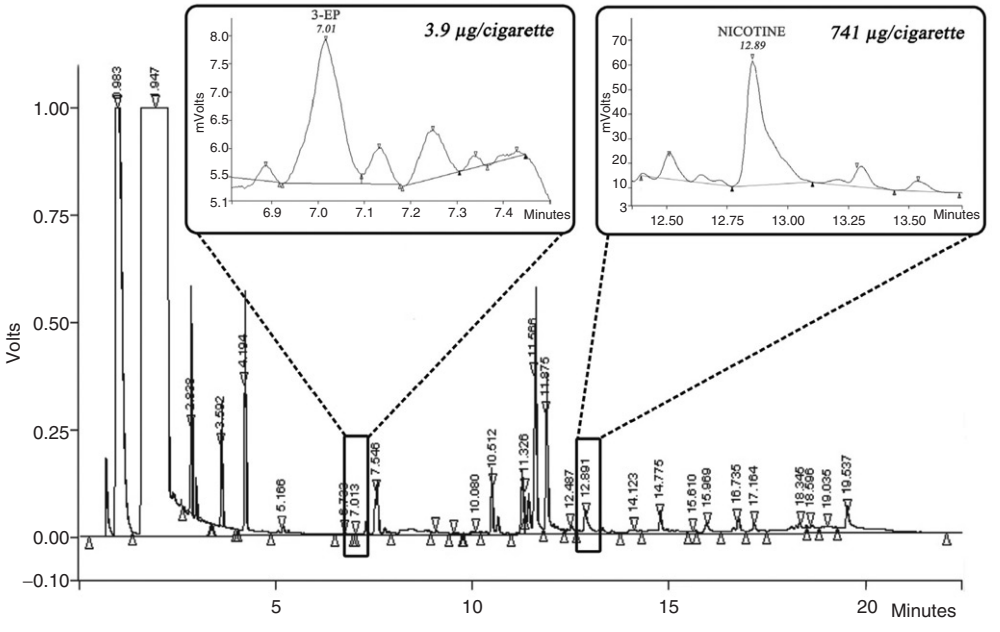

Figure 4. Exemplary chromatogram of tobacco smoke generated from reference cigarette with lowered nicotine content 3R4F (chromatography conditions described in the text).

Table 4. Results of method validation.

	Number of replicates	Nicotine	3-EP
Linearity	24	$r = 0.9969$	$r = 0.9971$
RSD	24	2.2%	1.9%
Inter-day precision	4 (per day)	7.2%	1.7%
Intra-day precision	28 (over a week)	9.8%	2.7%
Recovery	4	91.3%	95.4%
Accuracy	9	70.1%	87.3%
LOD	3 (calculated per one cigarette or	0.06 µg	0.06 µg
LOQ	1 m^3 of air polluted with ETS)	0.18 µg	0.19 µg

matrices, sample sizes, etc.). Moreover, the results of method validation were satisfactory compared with the ASTM [10], AOAC [11,12], US EPA [13] and ISO [5] methods (Table 5). LODs of the developed method were similar to LOD values of standard methods, which varies from 0.01 (ASTM) to 0.17 µg m^{-3} (US EPA) for nicotine and from 0.01 (ASTM) to 0.08 µg m^{-3} (ISO) for 3-EP. Analogically, our LOQ values were also acceptable in comparison with reference methods, which vary from 0.05 (ASTM) to 0.56 µg m^{-3} (ISO) for nicotine and from 0.02 (ASTM) to 0.28 µg m^{-3} (ISO) for 3-EP. Finally, the precision of the described method was within the range of values of the reference methods.

The previously described methods were designed to determine both or even one marker either in tobacco smoke or in indoor air. These methods are usually labour-intensive and

Table 5. Comparison of proposed method with ASTM, AOAC, US EPA and ISO standard methods for nicotine and 3-EP determination.

		(1-hour/8-hour sample duration)			
Source	Number	LOQ ($\mu g\,m^{-3}$)	LOD ($\mu g\,m^{-3}$)	Recovery	RSD
ASTM [10]	D 5075	Nicotine: 0.37/0.05 3-EP: 0.19/0.19	Nicotine: 0.11/0.01 3-EP: 0.06/0.01	Nicotine: 101.1% 3-EP: 100.8%	Nicotine: 2.2% 3-EP: 0.8%
AOAC [11]	990.01	n.s.	Nicotine: 0.06* 3-EP: 0.03*	Both: 100%	Nicotine: 7.5% 3-EP: 4.3%
AOAC [12]	991.50	n.s	Nicotine: 0.1*	Nicotine: 100%	Nicotine: 4.9%
US EPA [13]	IP-2	n.s.	Nicotine: 0.17/0.02	Nicotine: 100%	n.s.
ISO [14]	18145:2003	Nicotine: 0.56/0.07 3-EP: 0.28/0.03	Nicotine: 0.17/0.02 3-EP: 0.08/0.01	Nicotine: 100%	n.s.
Proposed method		Nicotine: 0.18** 3-EP: 0.19**	Nicotine: 0.06** 3-EP: 0.06**	Nicotine: 91.3% 3-EP: 95.4%	Nicotine: 2.2% 3-EP: 1.9%

Note: n.s.–not specified; *method requires 2-h sample period; **calculated per one cigarette or $1\,m^3$.

require the smoking of up to 20 cigarettes during a single trial instead of just one. Moreover, most of these methods are designed to determine nicotine and 3-EP in vapour and particulate phases of tobacco smoke separately. The vapour and particular levels are then combined in order to achieve the total amount of the compounds in tobacco smoke [14–19]. Application of the active sampling system described in the article does not require calibration of every single sampler each time when used by contrast with passive sampling systems.

Another advantage of our method is that it allows the determination of nicotine and 3-EP in one single cigarette. Some of the previously published methods required smoking a series of cigarettes [14,16]. The average value per cigarette is then calculated by dividing the final value for the marker by the number of cigarettes smoked. However, our last unpublished results and the results of Geiss and Kotzias [17] indicate that the differences in the concentration of both discussed ETS markers in MS and SS can be statistically significant among cigarettes of the same brand. Moreover, according to ISO standard for determination of nicotine in smoke condensates [15], it is necessary to smoke several cigarettes and this allows to determine nicotine in MS only. What is important, the ISO method requires a series of propan-2-ol traps, which can be inconvenient to operate in real conditions, such as experiments in pubs or apartments. Another formerly published method for nicotine determination in tobacco smoke [18] uses a series of methanol traps, and then distillation with water vapours. However, similarly to the ISO method, it can be used for nicotine determination in MS only.

The results presented in Table 1 indicate that the mean values of nicotine in MS are similar to those declared by cigarette manufacturers and determined by standard ISO procedures. For example, the level of nicotine determined by standard ISO method in reference cigarettes 3R4F is 730 μg per cigarette compared to our mean result of 735 μg per cigarette. However, SS levels of nicotine are significantly higher compared to data published by other authors [20]. The reason for this may be a higher SS/MS ratio (design of filter vents in the mouthpiece of modern cigarettes).

Our results of nicotine and 3-EP in indoor air (presented in Table 2) confirm that the compounds of interest occur at significant levels in rooms where people smoke cigarettes, and therefore can be used as markers of ETS. Hyvärien *et al.* [21] reported that the mean concentrations of nicotine in public places (discos, nightclubs, pubs) ranged from 1.4 to 42.2 $\mu g\,m^{-3}$, while 3-EP from 1.4 to 6.3 $\mu g\,m^{-3}$. Vainiotalo *et al.* [22] reported that mean concentrations of 3-EP in smoking environments ranged from 1.3 to 5.3 $\mu g\,m^{-3}$, although no 3-EP was detected in non-smoking rooms. In another study, Kuusimäki *et al.* [23] compared nicotine and 3-EP levels in smoking and non-smoking areas using passive sampling. The median concentrations of the discussed compounds were 1.5 and 11 $\mu g\,m^{-3}$ for 3-EP and nicotine, respectively. Moshammer *et al.* [24] conducted nicotine determinations in indoor air of public places like schools, restaurants and public means of transport using a passive and active sampling method. In total 106 locations were investigated, using active and passive nicotine sampling. The highest nicotine concentrations were found in discos (mean value: 154.4 with maximum of: 487.1 $\mu g\,m^{-3}$) while in public means of transport the concentrations were usually below 10 $\mu g\,m^{-3}$. It shows that the results obtained in various places may vary significantly and are influenced by many factors, which are often very hard to predict. The determined levels of nicotine and 3-EP in indoor air in our research are close to those obtained by other authors and the small differences in compounds levels may result from different conditions in the rooms where

the experiments were conducted. Such factors as the number of smokers, smoking frequency, room volume and its ventilation should be taken into consideration [25].

5. Conclusions

The developed method of nicotine determination in MS of one single cigarette can be applied for conducting a wide range of research projects concerning the diversification of active smokers' exposure to this toxic alkaloid, resulting from smoking various brands and types of cigarettes and also from an individual way of cigarette smoking. The method determines both markers in cigarette smoke from a single cigarette in order to control the diversification of nicotine and 3-EP levels in tobacco smoke generated from cigarettes of the same brand.

The method can also be used for the indirect monitoring of passive smokers' exposure to ETS. According to determined MS and SS levels of both markers, it would be possible to predict their concentration in a room where direct measurements can not be performed. However, other data required for such forecasting are: (1) number of smokers; (2) smoking frequency; (3) room volume and its ventilation. The authors plan to apply the method described during future evaluations of exposure prognosis models.

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