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Quantitative Estimation of Some Volatile N-Nitrosamines in Tobacco Smoke Using Validated GC-MS Method

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The aim of this work was the quantitative estimation of some volatile N-nitrosamines in tobacco smoke of local cigarette different brands using an efficient, rapid and sensitive GC-MS method. The chromatographic system suitability was tested by using the following characteristics. The RSD, % of peak areas (five replicate injections) was < 2.0 %; The RSD, % of retention times < 1.0 %; the number of theoretical plates was > 2000; the tailing factor < 2.0; the resolution between the two nearest peaks > 2.0 for all N-nitrosamines. The calibration curve was linear over a concentration range 0.5-100 µg mL^{-1} with a correlation coefficient > 0.99. The limit of detection and limit of quantitation were 0.25 and 0.5 µg mL^{-1}, respectively. The determined quantities of some volatile N-nitrosamines *e.g.*, N-nitrosodimethylamine, N-nitrosomethylethylamine and N-nitrosodiethylamine in tobacco smoke vary 190-320, 87-119 and 99-166 ng/cigarette, respectively.

Keywords: Volatile N-nitrosamine, GC-MS, Analytical method, Validation.

INTRODUCTION

Development of modern industry causes increasingly serious pollution in the environment where human lives in, constituting a catastrophic health risk including cancer. Anticancer is thus one of the challenges faced by the scientists in 21st century in the realm of life science and removal of carcinogen from environment is an important step. Nitrosamines are probably the most widespread carcinogens, existing in workplace, processed meats, cigarette smoke, cosmetics, pesticides, rubber products, beer and even are produced in the stomach by reaction of secondary amines and nitrite (NO_2^-) both taken from foods[1]. Nitrites are added to food as preservatives in meat and meat products preventing the Botulinus poisoning. Antioxidant food additives like vitamin C can prevent the formation of nitrosamines from nitrites[2]. Another source of nitrosamines is described by the reaction of nitrogen oxides with alkaloids as it is reported from the drying process of the germinated malt in beer production[3]. As nitrosamine levels in malt and beer have been significantly reduced in the brewing process, high analytical performance is required. In addition to the regular control of other food products for daily consumption, malt in beer is also monitored for low levels of nitrosamines. The first analytical studies on N-nitrosamines in tobacco smoke originated from the laboratory of Georg Neurath. N-nitrosamines in tobacco smoke originate from transfer from the tobacco into the smoke, from thermal degradation of nitrosamino acids and from pyrosynthesis during smoking[4]. There are more than one hundred publications describing the presence of volatile, non-volatile and tobacco-specific N-nitrosamines and N-nitrosamino acids in tobacco, tobacco smoke and environmental tobacco smoke.

The "classical" nitrosamine analysis was performed for many years by gas chromatography using a thermal energy analyzer as a detector. This special thermal energy analyzer detector was used due to its selectivity for nitrosamines with to the specific chemiluminescent reaction of ozone with the detector generated NO from nitrosamines. Today, with increased sensitivity requirements, the detection limits of the thermal energy analyzer and also its complex operation, no longer comply with the required needs for low detection limits and sample throughput. Also, several analytical methods have been employed in the past for the quantitative determination including colorimetry, spectrophotometry, polarography, capillary electro-chromatography, micellar electro-kinetic capillary chromatography, high performance liquid chromatography[5-9]. Chromato-Mass spectrometric methods have increasingly replaced the above-mentioned thermal energy analyzer [10-14].

The EPA method 521 by Munch and Bassett from 2004 provided at that time a suitable GC-MS method based on

chemical ionization (CI) using an ion trap mass spectrometer with internal ionization in contrast to ion trap mass spectrometers using a dedicated (external) ion source design. Current developments in GC-MS triple quadrupole technology deliver today high sensitivity and selectivity also in the small molecule mass range and allow the detection of nitrosamines at very low concentration levels even in complex matrix samples. This is made possible by using a much simpler and standard approach with the regular electron impact ionization (EI) for a very straightforward method for low level nitrosamine analysis[15].

The present work describes an efficient, sensitive and rapid method for routine detection and quantitation of volatile N-nitrosamines [nine volatile N-nitrosamines *i.e.*, N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (DPNA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMPA), N-nitrosodiphenylamine (NDPA)] diluted in methanol which was used to determine the abovementioned compounds in tobacco smoke of local different brands. Special focus in the method development has been made to provide the required high sensitivity for the detection of the nitrosamine compounds for a fast, easy to implement routine method. This study achieved satisfactory results in terms of linearity and precision under simple chromatographic conditions.

EXPERIMENTAL

EPA 8270 N-nitrosamine mix standard contained nine analytes in methanol at 2000 µg/mL of each: N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (DPNA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMPA), N-nitrosodiphenylamine (NDPA) was purchased from Supelco (USA). Solvent-methanol (GC grade) was purchased from Sigma-Aldrich (USA).

Instrumentation and methodology: The chromatographic analysis was performed using Agilent 6890 - Inert MSD 5975 Quadrupole GC-MS System (Agilent Technologies, USA). System control, data collection and data processing were accomplished using HP Chemstation software. The chromatographic condition was optimized using the Carbowax/20M (30 m × 0.25 mm × 0.25 µm) column; Gas carrier – He; Injection mode – splitless; Injection temperature – 220 °C; Volume – 1 µL; Oven program – 45 °C for 3 min (isocratic), then 20 °C/min to 220 °C (gradient) and 220 °C for 3.25 min for standard solution (total run time - 15 min) and 18.25 min (total run time – 30 min) for sample solution (isocratic); Average velocity – 36 cm sec^{-1}; Flow rate – 1.0 mL min^{-1}, constant flow; Ionization mode – El; Mass resolution setting – normal; Source temperature - 230 °C. The statistical analysis and the evaluation of uncertainty of analytical procedure were performed using Microsoft Excel 2010 according to NATA, ISO, EUROLAB guidelines[16,17]. The method validation was performed according to ICH and Eurachem guidelines[18-20].

Preparation of solutions: Standard solution - 0.25 mL of 2000 µg mL^{-1} N-nitrosamines mix standard was accurately measured and transferred to a 10 mL volumetric flask and was diluted up to the mark with the diluent (methanol). Then it was mixed well and filtered through 0.45 µm syringe filter (50 µg mL^{-1}).

Sample solution: This method can be used to determine volatile N-nitrosamines diluted in methanol as a sample solution, which can be obtained from tobacco smoke or solid/liquid material using extraction. The concentration of sample solution should not be less than 0.5 µg mL^{-1} for each N-nitrosamine. This method was used to determine volatile N-nitrosamines in tobacco smoke. Sample solutions were prepared using specially constructed laboratory instrument which was composed of the following parts: 1.Specially made quartz tube for burning tobacco; 2. Specially made glassware with bubbler on glacial bath for N-nitrosamine absorption; 3.Vacuum pump. The smoke from tobacco burning in quartz tube was conducted through solvent which absorbs all N-nitrosamines without any loses. The obtained sample solution was filtered through 0.45 µm syringe filter.

The standard and sample solutions were prepared in dark glassware, protected from light and were analyzed immediately. The standard solutions were stored in refrigerator during analysis.

Quantitative estimation of N-nitrosamine: The concentration (C_u), µg mL^{-1} of N-nitrosamine in sample solution was calculated by the formula:

$$C_u = \frac{A_u \times C_s \times V \times P}{A_s \times 10} \times 100$$

where, A_u - Peak area of N-nitrosamine obtained from the chromatogram of sample solution; A_s - Peak area of N-nitrosamine obtained from the chromatogram of standard solution; C_s - The concentration of N-nitrosamine in standard, µg mL^{-1}; V - The volume of standard, mL; P - Purity of standard, %.

The quantity (X), µg/cigarette of each N-nitrosamine in tobacco smoke was calculated by the formula:

$$X = \frac{C_u \times W_c \times V}{W_T}$$

where, C_u - determined concentration (µg mL^{-1}) of N-nitrosamine in sample solution; W_c – average mass of weighed cigarette (mg, calculated on 20 units); V – volume of solvent (mL) (methanol); W_T – mass of weighed tobacco (mg).

Method validation

Linearity and range: From stock solution (100 µg mL^{-1}) standard working solutions of N-nitrosamines were prepared at seven different concentration levels ranging from 0.5-100 µg mL^{-1} (0.5, 1, 10, 12.5, 25, 50, 100 µg mL^{-1}) for all compounds. Three replicate injections (n = 3) were performed at each concentration of N-nitrosamine. The linearity was checked by the correlation coefficient (acceptance criteria: > 0.99), the square of correlation coefficient (acceptance criteria: > 0.98), the Y-intercept, % (acceptance criteria: < 5.0 %), the RSD, % (relative standard deviation) of retention times (acceptance criteria: < 1.0 %).

Limit of quantitation (LOQ) and limit of detection (LOD): The signal-to-noise ratio (s/N) of method was adopted

for the determination of the lower limit of quantitation/lower limit of detection. The limit of quantitation was estimated to be ten times the S/N ratio. The limit of detection was estimated to be three times of S/N ratio (acceptance criteria). The quantitation limit was achieved by injecting a series of possible dilute solutions of all components and the precision was established at the quantitation level. The RSD, % of peak areas for LOQ should not be more than 10.0 % and the RSD, % of retention times for both lower limits should not be more than 1.0 %.

System suitability test: The system suitability parameters were measured to verify the chromatographic system performance. System suitability was checked by five replicate injections (n = 5) of standard solution. Main parameters including the RSD, % of peak areas (acceptance criteria: < 2.0 %), the RSD, % of retention times (acceptance criteria: < 1.0 %), the resolution between all the nearest peaks (acceptance criteria: > 2.0), the tailing factor (acceptance criteria: < 2.0) and the number of theoretical plates (acceptance criteria: > 2000) were measured.

Precision: The precision was estimated by measuring repeatability and time-dependent intermediate precision on five replicate injections of standard solution and on three individual determination of N-nitrosamines in sample solution. The precision was checked by the RSD, % of determined concentrations (μg mL^{-1}) and the RSD, % of retention times for three individual determinations of N-nitrosamines which should not be more than 10 % and 1 %, respectively, also by the percentage difference, (%) between two inter-day determinations of N-nitrosamines which should not be more than expanded uncertainty value (acceptance criteria).

Robustness: The robustness test examines the effect that operational parameters have on the analysis results. For determination of a method's robustness a number of method parameters, for example standard solution stability is varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. In this study, only one factor - standard solution stability was evaluated during 4 days stored in dark glassware under refrigeration, protected from light. The stability of the solution was studied initially, after 1, 2, 4, 6, 24 h and 2, 4 days against freshly prepared standard solution. The stability was checked by the percentage difference; % between peak areas of standard solutions stored in refrigerator and freshly prepared which should not be more than 3 % (acceptance criteria).

Uncertainty estimation: In order to obtain an estimate of the uncertainty associated with a measurement result the following tasks were needed to be performed: to specify the measurement; to identify the sources of uncertainty; to calculate the uncertainty components associated with each potential source of uncertainty identified; to calculate the standard uncertainty, applying the appropriate coverage factor, to give an expanded uncertainty. The following sources of uncertainty were identified: analytical balance, repeatability, equipment, measuring glassware, measuring pipette. It was estimated uncertainties of solution preparation and analytical procedure (repeatability measurement), separately.

RESULTS AND DISCUSSION

Linearity and range: For all the compounds, the plotted linearity graphs were straight line over the range from 0.5-100 μg mL^{-1} (1-7 level), the correlation coefficients were greater than 0.99; the Y-intercepts, % were less than 5 %; The RSD, % of retention times of each N-nitrosamine in 3 replicate injections was less than 1 % (0.003-0.096 %); The linearity concentration and regression statistics are shown in Table-1 for 3 N-nitrosamines (NDMA, NMEA, NDEA). The linearity (calibration) graphs are presented in Figs. 1-3.

Limit of quantitation (LOQ) and limit of detection (LOD): The determined lower limit of quantitation and precision at LOQ values for all components are presented in Table-2. The LOQ of the method was estimated to be equal to 0.5 μg mL^{-1} and 0.25 μg mL^{-1} could be considered as the LOD according to the acceptance criteria. Fig. 4 shows the chromatogram of 50 μg mL^{-1} standard solution (100 %).

System suitability test: The RSD (%) of peak areas for all N-nitrosamine were below 2.0 %. The RSD (%) of retention times was below 1.0 %. The resolution between the two nearest peaks was more than 2.0. The tailing factor was less than 2.0. The number of theoretical plates was more than 2000. These indicate that the chromatographic system is suitable for determination of all nine N-nitrosamine compounds. The system suitability test results are given in Tables 3 and 4.

TABLE-1
REGRESSION STATISTICS FOR N-NITROSODIMETHYLAMINE (NDMA) (PURITY 99.9 %), N-NITROSOMETHYLETHYLAMINE (NMEA) (PURITY 99.8 %) AND N-NITROSODIETHYLAMINE (NDEA) (PURITY 99.9 %)

Level	NDMA		NMEA		NDEA	
	Concentration (μg mL^{-1})	Average peak area (n = 3)	Concentration (μg mL^{-1})	Average peak area (n = 3)	Concentration (μg mL^{-1})	Average peak area (n = 3)
1	99.90	146687436	99.80	193771256	99.90	245614223
2	49.95	74215506	49.90	97892749	49.95	124177784
3	24.98	35972792	24.95	52210384	24.98	63369197
4	12.49	17527844	12.48	27397807	12.49	33017830
5	9.990	14245705	9.980	22556458	9.990	27239937
6	0.998	1424571	0.998	2234645	0.998	2625783
7	0.499	712578	0.499	1025445	0.499	1474568
Correlation coefficient (r)	0.99995		0.99974		0.99993	
Square of correlation coefficient (r^2)	0.99990		0.99947		0.99985	
Slope	1475471		1927315		2448888	
Y-Intercept	320718		2045741		1525432	
Y-Intercept (%)	0.43		2.09		1.23	

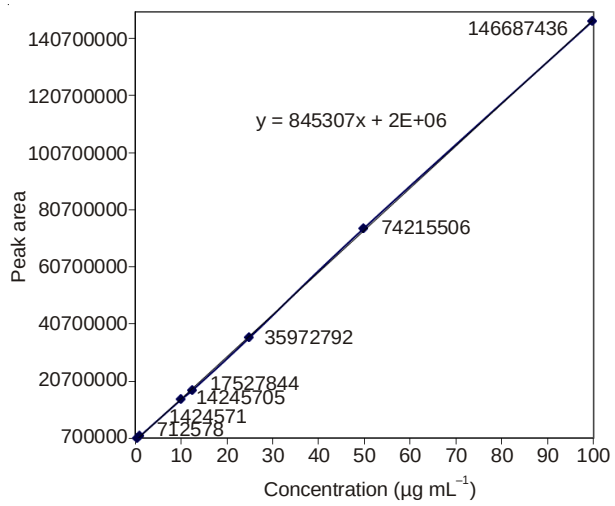

Fig. 1. Linearity graph of N-nitrosodimethylamine (NDMA)

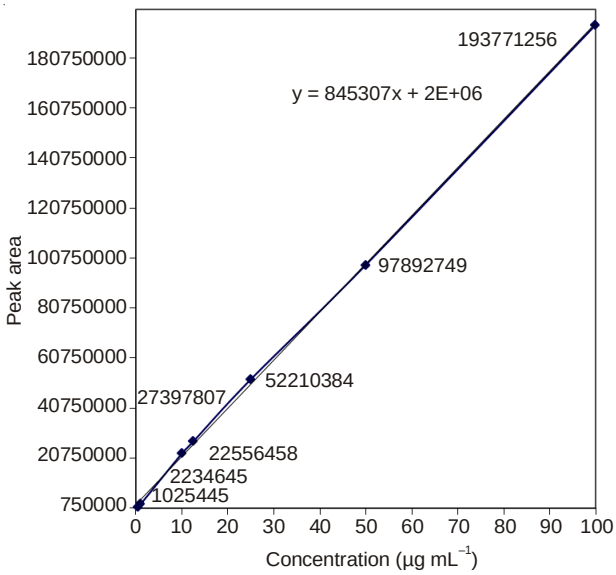

Fig. 2. Linearity graph of N-nitrosomethylethylamine (NMEA)

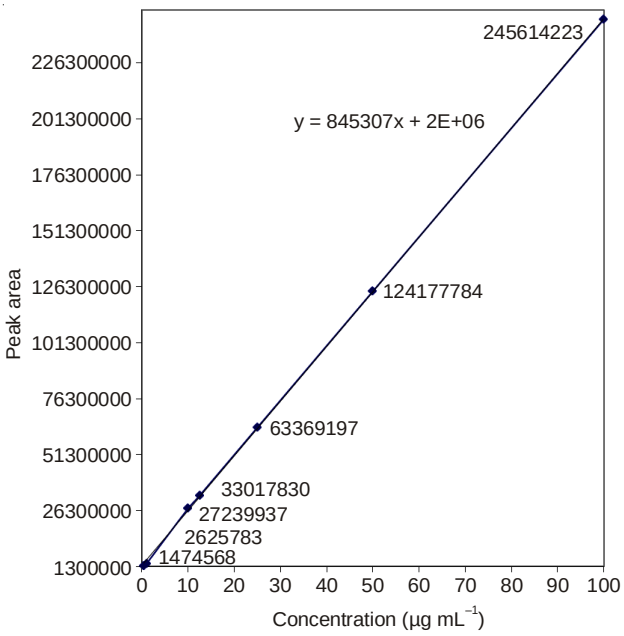

Fig. 3. Linearity graph of N-nitrosodiethylamine (NDEA)

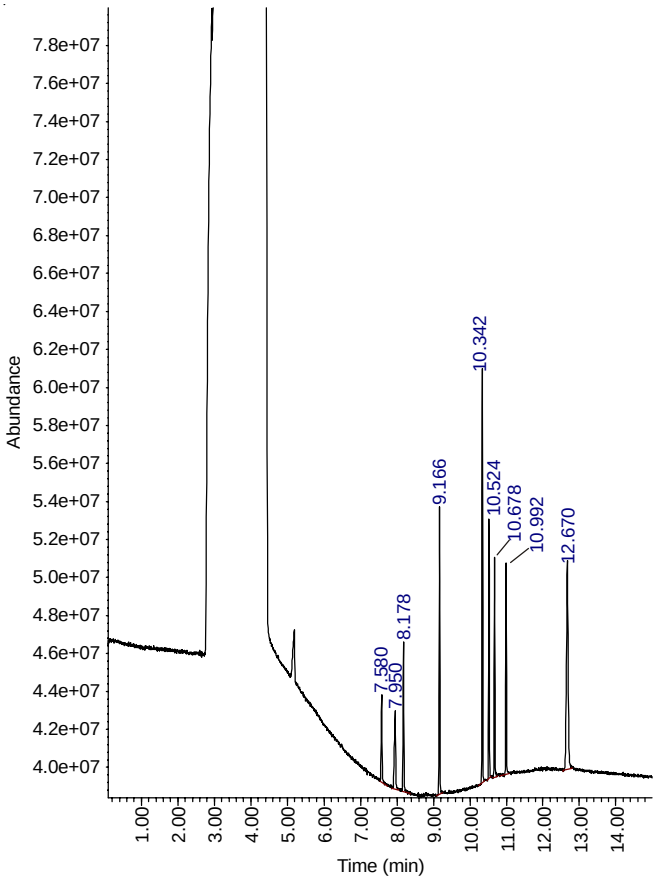

Fig. 4. Chromatogram of 50 µg mL^{-1} standard solution: Retention time (RT), in minutes: 7.580 - N-nitrosodimethylamine (NDMA), 7.950 - N-nitrosomethylethylamine (NMEA), 8.178 - N-nitrosodiethylamine (NDEA), 9.166 - N-nitrosodipropylamine (DPNA), 10.342 - N-nitrosodibutylamine (NDBA), 10.524 - N-nitrosopiperidine (NPIP), 10.676 - N-nitrosopyrrolidine (NPYR), 10.992 - N-nitrosomorpholine (NMPA), 12.670 - N-nitrosodiphenylamine (NDPA)

Precision: The precision results (Table-5) show that the calculated RSD (%) of determined concentrations (three individual determinations) in sample solutions for each N-nitrosamine and the percentage difference (%) between two inter-day determinations for each N-nitrosamine comply with the acceptance criteria. The calculated RSD (%) of retention times was below 1.0 % (0.005 % - 0.396 %) for each N-nitrosamine.

Robustness: The stability of standard solution after 6, 24 h and 4 days (under refrigeration), protected from light is shown in Table-6. Standard solution of N-nitrosamines is stable for the period up to 6 h under refrigeration stored in dark glassware, protected from light.

Uncertainty estimation: The results of estimation of uncertainty on the examples of N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA) and N-nitrosodiethylamine (NDEA) are shown in Tables 7-9. The uncertainty value was used as acceptance criteria for evaluation the method precision, more precisely, the percentage difference, % between two inter-day determinations of each N-nitrosamine should not be more than expanded uncertainty value.

Determination of N-nitrosamines content in cigarette: The determined quantities of N-nitrosamines in tobacco smoke of different local brands are shown in Table-10.

TABLE-2
LIMIT OF QUANTITATION (LOQ) AND LIMIT OF DETECTION (LOD) FOR EACH N-NITROSAMINE

	NDMA	NMEA	NDEA	DPNA	NDBA	NPIP	NPYP	NMPA	NDPA
Purity (%)	99.90	99.80	99.90	99.90	99.90	99.90	99.90	99.90	96.58
LOQ (μg mL^{-1})	0.500	0.499	0.500	0.500	0.500	0.500	0.500	0.500	0.4823
LOD (μg mL^{-1})	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.242
RSD (%) of peak areas for LOQ (n = 3)	8.182	7.814	8.452	5.412	9.012	6.541	7.774	8.412	8.001
RSD (%) of peak areas for LOD (n = 3)	16.251	13.256	12.454	14.7891	16.475	13.256	11.246	13.471	10.241
RSD (%) of retention times for LOQ (n = 3)	0.008	0.010	0.006	0.041	0.003	0.005	0.029	0.014	0.444
RSD (%) of retention times for LOD (n = 3)	0.060	0.020	0.005	0.057	0.050	0.100	0.098	0.043	0.354
S/N for LOQ	11.5	11.9	13.0	18.3	19.6	14.9	15.1	16.5	13.9
S/N for LOD	3.1	3.6	4.4	7.4	7.5	5.5	6.8	6.4	4.2

TABLE-3
RSD (%) OF PEAK AREAS (n = 5) OBTAINED FROM THE 50 μg mL^{-1} STANDARD SOLUTION CHROMATOGRAMS

Injection #	NDMA	NMEA	NDEA	DPNA	NDBA	NPIP	NPYP	NMPA	NDPA
1	75556574	100343457	117827730	203714982	287416622	201616533	169226105	165868312	360192767
2	74447864	100300025	117458711	202145023	286417831	201499704	168206245	165458400	359254325
3	74317865	97465435	114857169	201789452	285687621	197516531	168126175	159948635	359143745
4	73339845	97745364	113114078	203714982	285512560	197216500	167811325	159728974	359145700
5	73312436	97140244	113817731	203714982	285378142	198016571	167424076	159778134	358717653
Average	74194917	98598905	115415084	203015884	286082555	199173168	168158785	162156491	359290838
RSD (%)	1.250	1.610	1.846	0.476	0.296	1.103	0.399	1.977	0.152

TABLE-4
RSD (%) OF RETENTION TIMES (n = 5) OBTAINED FROM THE 50 μg mL^{-1} STANDARD SOLUTION CHROMATOGRAMS

Injection #	NDMA	NMEA	NDEA	DPNA	NDBA	NPIP	NPYP	NMPA	NDPA
1	7.580	7.950	8.178	9.166	10.342	10.524	10.676	10.992	12.670
2	7.579	7.951	8.178	9.166	10.341	10.523	10.676	10.991	12.513
3	7.580	7.951	8.179	9.167	10.342	10.523	10.675	10.995	12.514
4	7.580	7.952	8.178	9.167	10.342	10.524	10.682	10.992	12.511
5	7.580	7.950	8.179	9.156	10.342	10.524	10.676	10.995	12.514
Average	7.580	7.951	8.178	9.164	10.342	10.524	10.677	10.993	12.544
RSD (%)	0.006	0.011	0.007	0.052	0.004	0.005	0.026	0.017	0.560

TABLE-5
PRECISION RESULTS FOR N-NITROSODIMETHYLAMINE (NDMA), N-NITROSOMETHYLETHYLAMINE (NMEA) AND N-NITROSODIETHYLAMINE (NDEA)

Sample solution #	Concentration (μg mL^{-1})					
	NDMA		NMEA		NDEA	
	I day	II day	I day	II day	I day	II day
1	1.654	1.862	0.600	0.701	0.850	0.864
2	1.492	1.716	0.607	0.607	0.730	0.866
3	1.638	1.682	0.519	0.641	0.793	0.995
Average	1.595	1.753	0.575	0.650	0.791	0.908
RSD (%)	5.589	5.435	8.468	7.357	7.597	8.244
Percentage difference, %	9.44		12.24		13.77	

TABLE-6
STABILITY OF STANDARD SOLUTION

Time	Peak area of N-nitrosamine								
	NDMA	NMEA	NDEA	DPNA	NDBA	NPIP	NPYP	NMPA	NDPA
Freshly prepared	75556574	100343457	117827730	203714982	287416622	201616533	169226105	165868312	360192767
After 6 h	73525684	98586456	115871969	199725435	281914156	196456325	168695652	161981432	353684522
Difference (%)	2.72	1.77	1.67	1.98	1.93	2.59	0.31	2.37	1.82
After 24 h	54621724	71811825	90864086	154718206	215380581	150880748	117514680	119559422	266792897
Difference (%)	32.16	33.15	25.84	27.34	28.65	28.79	36.07	32.45	29.79
After 4 days	N.D.	57337871	66710508	107673910	157304281	116059489	93370476	83813572	N.D.
Difference (%)	-	54.55	55.40	61.69	58.51	58.86	57.77	65.73	-

N.D. = Not detected

TABLE-7
UNCERTAINTY'S BUDGET OF SOLUTION PREPARATION

Expanded uncertainty of solution preparation										
Source	#	Component	Value	Deviation	Unit	Type of uncertainty	Degree of freedom (f)	Probability ($P_{1,\%}$)	Probability distribution factor (k)	Standard uncertainty (u_i, %)
Standard solution	1	0.5 mL glass pipette	0.25	0.005	mL	B	∞	100	1.73	1.1557
	2	10 mL measuring flask	10	0.025	mL	B	∞	100	1.73	0.1443
Sample solution	3	5 mL pipette	5	0.030	mL	B	∞	100	1.73	0.3464
	4	Balance - Sartorius LE 323S -OCE	16650	0.100	mg	B	∞	95	1.73	0.0003

TABLE-8
UNCERTAINTY'S BUDGET OF ANALYTICAL PROCEDURE

Expanded uncertainty of analytical procedure												
Source	#	Component-measuring equipment	N-nitrosamine	RSD of peak areas (%)	Injection number (n)	Number of solution (m)	Type of uncertainty	Degree of freedom (f)	Student coefficient - t (f; P_1 %)	Probability ($P_{1,\%}$)	Probability distribution factor (k)	Standard uncertainty (u_i, %)
Standard solution	1	Agilent GC-MS System	NDMA	1.250	5	1	A	4	2.132	95	2.00	1.1917
			NMEA	1.610	5	1	A	4	2.132	95	2.00	1.5349
			NDEA	1.846	5	1	A	4	2.132	95	2.00	1.7599
Sample solution	2	Agilent GC-MS System	NDMA	5.589	3	3	A	6	1.943	95	2.00	6.2703
			NMEA	8.468	3	3	A	6	1.943	95	2.00	9.5003
			NDEA	7.597	3	3	A	6	1.943	95	2.00	8.5231

TABLE-9
UNCERTAINTY ESTIMATION RESULTS

N-nitrosamine	Combined standard uncertainty of solution preparation (u_{SP}, %)	Expanded uncertainty of solution preparation (U_{SP}, %)	Combined standard uncertainty of analytical procedure (u_{AP}, %)	Expanded uncertainty of analytical procedure (U_{AP}, %)	Expanded uncertainty (U, %)
NDMA	1.21	2.10	6.38	12.77	12.94
NMEA	1.21	2.10	9.62	19.25	19.36
NDEA	1.21	2.10	8.70	17.41	17.53

TABLE-10
CALCULATED QUANTITIES OF N-NITROSAMINES (N-NITROSODIMETHYLAMINE (NDMA), N-NITROSOMETHYLETHYLAMINE (NMEA) AND N-NITROSODIETHYLAMINE (NDEA) (ng/cigarette)

Sample #	Quantity of N-nitrosamine (ng/cigarette)					
	NDMA		NMEA		NDEA	
	Brand 1	Brand 2	Brand 1	Brand 2	Brand 1	Brand 2
1	280	190	110	90	144	108
2	320	236	119	87	166	99
Average	300	213	115	89	155	104

Conclusion

It has been determined the content of some volatile N-nitrosamines in tobacco smoke of local different brands using a rapid and sensitive GC-MS method which has been validated with respect to precision, linearity, limit of detection and limit of quantitation, robustness (standard solution stability). This method can be used to apply successfully for routine analysis in environmental and food safety monitoring laboratories for quantitative determination of nine volatile N-nitrosamines in methanolic sample solutions.

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