Eurasian Journal of Analytical Chemistry

Eurasian J Anal Chem 9(2): 79-91, 2014

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

Imeda Rubashvili*

Petre Melikishvili Physical and Organic Chemistry, Ivane Javakhishvili Tbilisi State University, 0186, Anna Politkovskaya Str., Tbilisi, Georgia.

Received: *07/12/2014****; Accepted:*** *27/12/2014*

Abstract

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 which was validated before. 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 LOD and LOQ were 0.25 µg mL^{-1} and 0.5 µg mL^{-1}, respectively.

This method can be used to determine nine volatile N-nitrosamines namely N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosopyrrolidine (NPYR), N-nitrosomorpholine (NMPA), N-nitrosodiphenylamine (NDPA) diluted in solvent - methanol as a sample solution which can be obtained from tobacco smoke or solid/liquid material using extraction. The determined quantities of some volatile N-nitrosamines - NDMA, NMEA and NDEA in tobacco smoke vary 190 - 320, 87 – 119 and 99 – 166 ng/cigarette, respectively.

Keywords:

Volatile N-nitrosamine, GC-MS, Analytical Method Validation

1. Introduction

Development of modern industry causes increasingly serious pollution in the environment where human lives in, constituting a catastrophic health risk including cancer. Anti-cancer is thus one of the challenges faced 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

* **Corresponding Author**
E-mail: rubashvili@yahoo.fr
ISSN: 1306-3057

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 is more than one hundred publications have described 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 (TEA) as detector. This special TEA detector was used due to its selectivity for nitrosamines with to the specific chemiluminiscent reaction of ozone with the detector generated NO from nitrosamines. Today, with increased sensitivity requirements, the detection limits of the TEA, 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 TEA [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 very 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 - N-nitrosodimethylamine - NDMA, N-nitrosomethylethylamine - NMEA, N-nitrosodiethylamine - NDEA, N-nitrosodipropylamine - NDPA, N-nitrosodibutylamine - NDBA, N-nitrosopiperidine - NPIP, N-nitrosopyrrolidine - NPYR, N-nitrosomorpholine - NMPA, N-nitrosodiphenylamine – NDPA) diluted in methanol which was used to determine the above-mentioned 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.

2. **Experimental**

2.1. Chemical and Reagents

EPA 8270 N-nitrosamine mix standard contained nine analytes in methanol at 2000 µg/mL of each: N-nitrosodimethylamine-NDMA, N-nitrosomethylethylamine-NMEA, N-nitro-sodiethylamine - NDEA, N-nitrosodipropylamine - NDPA, N-nitrosodibutylamine - NDBA, N-nitrosopiperidine - NPIP, N-nitrosopyrrolidine - NPYR, N-nitrosomorpholine - NMPA, N-nitrosodiphenylamine – NDPA and individual standards to each of the N-nitrosamines with a concentration of 5000 µg/mL in methanol were purchased from Supelco

(USA). For sample preparation solvent – methanol (GC grade) was purchased from Sigma-Aldrich (USA).

2.2. Instrumentation and methodology

The chromatography 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 x 0.25 mm x 0.25µm) column; Gas carrier – He; Injection mode – splitless; Injection temperature – 220^0C; Volume - 1µL; Oven program - 45^0C for 3 min (isocratic), then 20^0C/min to 220^0C (gradient) and 220^0C 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; Total run time – 15 min for standard solution and 30 min for sample solution; Ionization mode – El; Mass resolution setting – normal; Source temperature - 230^0C. The statistical analysis and the evaluation of uncertainty of analytical procedure were performed using Microsoft Excel 2010 according to NATA, ISO, EUROLAB guidelines [16-19]. The method validation was performed according to ICH and Eurachem guidelines [20-22].

2.3. Preparation of standard and sample solutions

2.3.1. 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}).

2.3.2. 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 1.0 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 (as a solvent was used methanol); 3.Vacuum pump. The smoke from tobacco burning in quartz tube was conducted trough solvent which absorbs all N-nitrosamine compounds 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 analysed immediately. The standard solutions were stored in refrigerator during analysis.

2.4. Quantitative estimation of N-nitrosamine (External standard method)

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

$$C_U = \frac{A_U x C_S x V x P}{A_S x 10 x 100}$$

Where, Au - Peak area of N-nitrosamine obtained from the chromatogram of sample solution; As - 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:

$$C_U = \frac{C_U x W_C x V}{W_T}$$

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

2.5. Method validation

2.5.1. Linearity and range

The linearity of an analytical method is its ability to elicit results that are directly or by a well-defined mathematical transformation, proportional to the concentration of the analyte in a sample within a given 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 %).

2.5.2. Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The LOD is the smallest quantity of the targeted substance that can be detected but not accurately quantified in the sample. The LOQ of method is the lowest amount of the targeted substance, which can be quantitatively determined under the experimental conditions prescribed with included inside the acceptance limits over the concentration range investigated. The signal-to-noise ratio (s/N) of method was adopted for the determination of the lower limit of quantitation. The limit of quantitation is estimated to be ten times the s/N ratio; the limit of detection is 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 %.

2.5.3.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.

2.5.4. Precision

The precision of an analytical method is the degree of agreement among the individual test results obtained, when the method is repeated with multiple samples from the same homogeneous sample mix. It 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 10.0 % and 1.0 %, 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).

2.5.5. 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 hours 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 3.0 % (acceptance criteria).

3. Results and Discussion

3.1. Validation parameters

3.1.1. 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 5.0 %; The RSD, % of retention times of each N-nitrosamine in 3 replicate injections was less than 1.0 % (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 Figure 1, 2, 3.

Table 1. The 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	9.99	14245705	9.98	22556458	9.99	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	

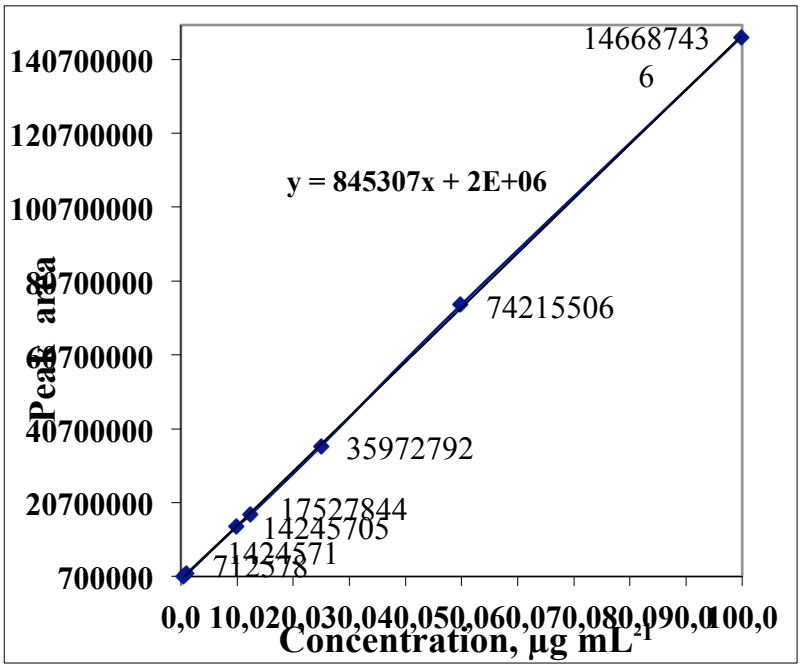

Fig. 1. The linearity (calibration) graph of N-nitrosodimethylamine (NDMA)

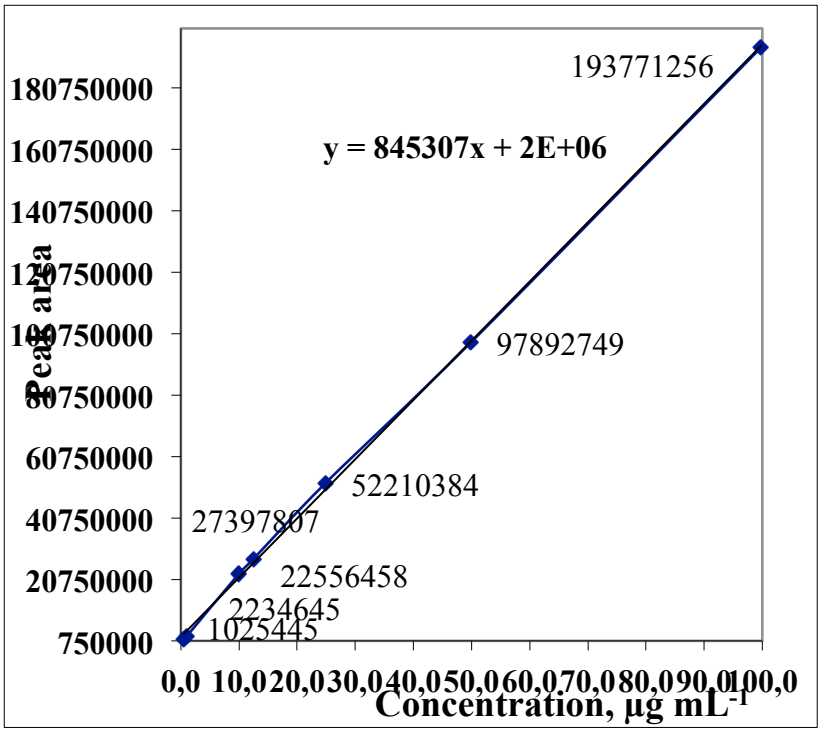

Fig. 2. The linearity (calibration) graph of N-nitrosodimethylamine (NMEA)

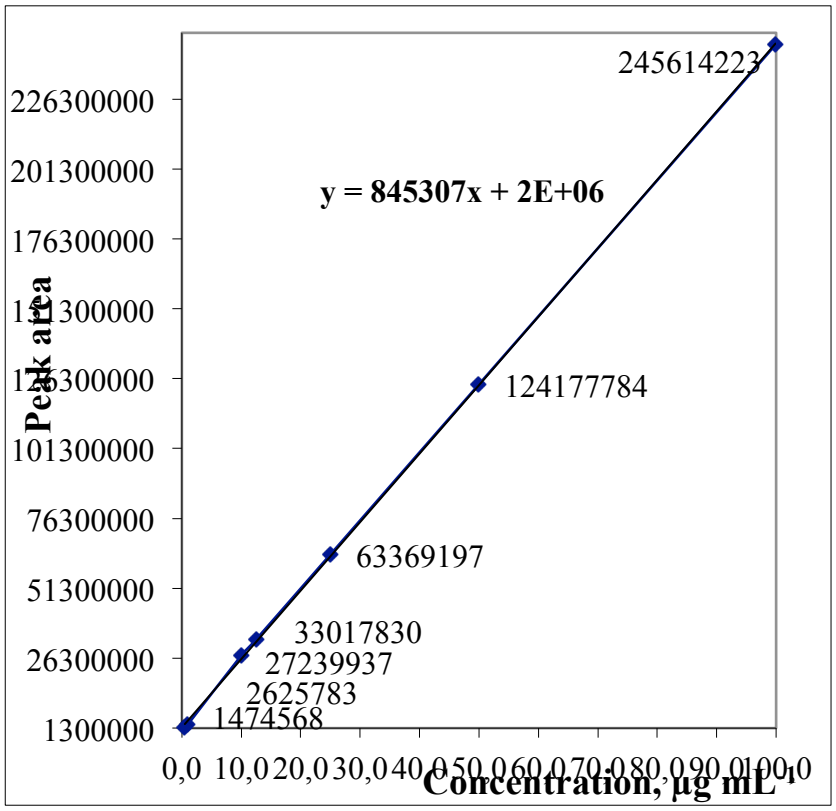

Fig. 3. The linearity (calibration) graph of N-nitrosodiethylamine (NDEA)

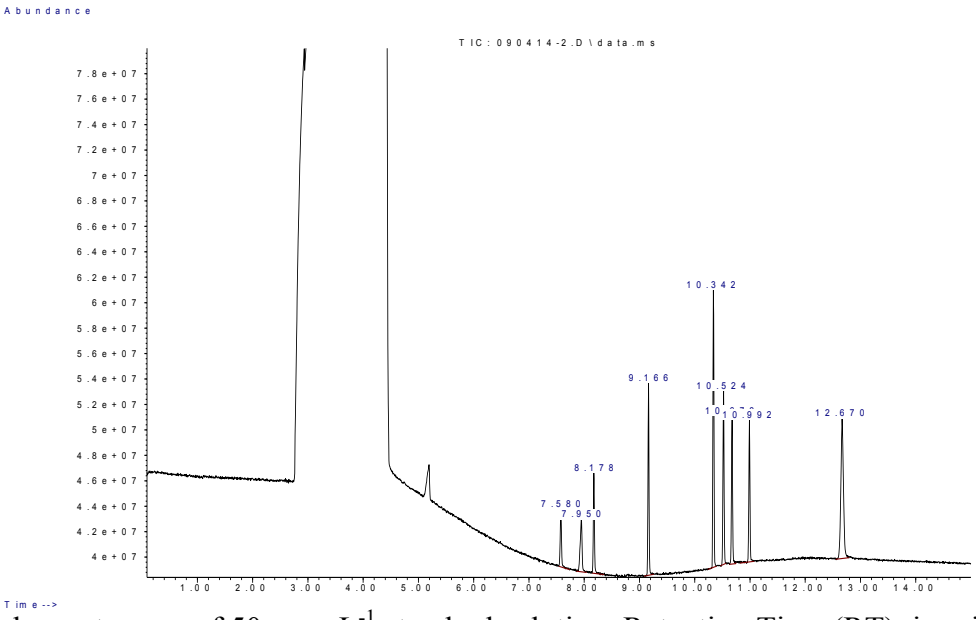

Fig. 4. The chromatogram of 50 µg mL^{-1} standard solution: Retention Time (RT), in minutes: 7.580 - N-nitrosodimethylamine - NDMA, 7.950 - N-nitrosomethylethylamine - NMEA, 8.178 - N-nitrosodiethylamine - NDEA, 9.166 - N-nitrosodipropylamine - NDPA, 10.342 - N-nitrosodibutylamine - NDBA, 10.524-N-nitrosopiperidine-NPIP,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

3.1.2. 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, 5 shows the chromatograms of 50 µg mL^{-1} (100 %) and 0.25 µg mL^{-1} (LOD) standard solutions, respectively.

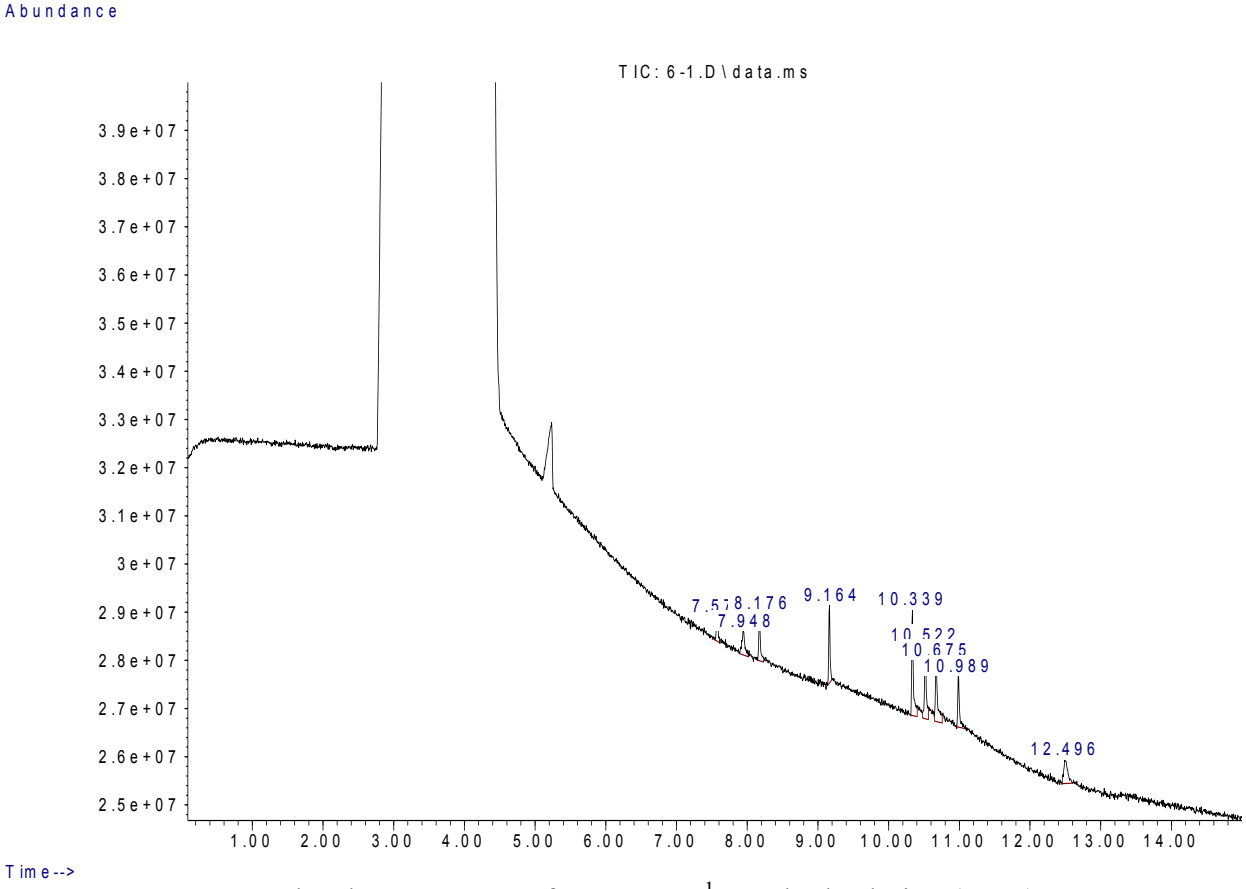

Fig. 5. The chromatogram of 0.5 µg mL^{-1} standard solution (LOQ)

Table 2. LOQ and LOD for each N-nitrosamine

3.1.3. System suitability test

The RSD, % of peak areas for all N-nitrosamine was below 2.0 %; The RSD, % of retention times – 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 Table 3, 4.

3.1.4. 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.

3.1.5. Robustness

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

Table 2. LOQ and LOD for each N-nitrosamine

Abbreviation	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	0.499	0.250	0.250	0.250	0.250	0.250	0.250	0.242
The 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
The 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
The 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
The 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. The 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. The 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. The precision results for N-nitrosodimethylamine (NDMA), N-nitrosomethylethyl-amine (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. The stability of standard solution

Time	The 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 hours	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 hours	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	Not detected	57337871	66710508	107673910	157304281	116059489	93370476	83813572	Not detected
Difference, %	-	54.55	55.40	61.69	58.51	58.86	57.77	65.73	-

3.2. Uncertainty estimation

In order to obtain an estimate of the uncertainty associated with a measurement result the following tasks were need to be performed: to specify the measurand; 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. I was estimated uncertainties of solution preparation and repeatability, separately. The results of estimation of uncertainty on example of N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA) and N-nitrosodiethylamine (NDEA) are shown in Table 7.

Table 7. The expanded uncertainty's budget illustrated by quantitative determination of N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA) and N-nitrosodiethylamine (NDEA)

Expanded uncertainty of solution preparation														
Source	**#**	**Component**	**Value**	**Deviation**	**Unit**	**Type of uncertainty**	**Degree of freedom - f**	**Probability - $P_{1,\%}$**	**Probability distribution factor- k**	**Sensitivity Coefficient - c**	**Standard uncertainty - u , %**	**Component of uncertainty - c*u**	**Expanded uncertainty - U, %**
Standard solution	1	0.5 mL glass pipette	0.25	0.005	mL	B	10	100	1.73	1	2.00	2.00	3.460
	2	10 mL measuring flask	10	0.025	mL	B	10	100	1.73	1	0.25	0.25	0.433
Sample solution	3	5 mL pipette	5	0.030	mL	B	10	100	1.73	1	0.60	0.60	0.104
	4	Balance - Sartorius LE 323S -OCE	16650	0.100	mg	B	10	95	2.00	1	0.0006	0.0006	0.001
Expanded uncertainty of solution preparation, U_{SP} %													3.488

Expanded uncertainty of repeatability measurement															
Source	**#**	**Component**	**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**	**Sensitivity Coefficient - c**	**Standard uncertainty - u , %**	**Component of uncertainty - c*u**	**Expanded uncertainty - U, %**
Standard solution	**1**	Agilent 6890 - Inert MSD 5975 Quadrupole GC-MS System	NDMA	1.250	5	1	A	4	2.132	95	2.00	1	1.192	1.192	2.383
			NMEA	1.610	5	1	A	4	2.132	95	2.00	1	1.535	1.535	3.070
			NDEA	1.846	5	1	A	4	2.132	95	2.00	1	1.760	1.760	3.520
Sample solution	**2**		NDMA	5.589	3	3	A	6	1.943	95	2.00	1	6.270	6.270	12.541
			NMEA	8.463	3	3	A	6	1.943	95	2.00	1	9.495	9.495	18.989
			NDEA	7.597	3	3	A	6	1.943	95	2.00	1	8.523	8.523	17.046
Expanded uncertainty of repeatability measurement, Urm %															
NDMA															12.765
NMEA															19.236
NDEA															17.406
Expanded uncertainty, U %															
NDMA															13.233
NMEA															19.550
NDEA															17.752

Determination of N-nitrosamines content in cigarette

The determined quantities of N-nitrosamines in tobacco smoke of local different brands are shown in Table 8.

Table 8. The 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

4. Conclusion

It has been determined some volatile N-nitrosamines content 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 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.

Acknowledgments

The author is thankful to the management of Petre Melikishvili institute of physical and organic chemistry of Iv. Javakhishvili Tbilisi State University for providing facilities to carry out this work. This project is financially supported by the Shota Rustaveli National Science Foundation within the president grant for young scientists, Georgia.

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