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Improved Method for the Determination of Tobacco-specific Nitrosamines (TSNA) in Tobacco Smoke*

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Dedicated to Prof. Dr. R. Preussmann on the occasion of his 60th birthday.

SUMMARY

The method used to determine tobacco-specific nitrosamines [TSNA], *N'*-nitrosonornicotine [NNN], 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], *N'*-nitrosoanabasine [NAB] and *N'*-nitrosoanatabine [NAT], in mainstream smoke was modified. The methodology included trapping mainstream smoke in a buffer solution and on a Cambridge filter, extracting TSNA from the buffer solution and the filter with dichloromethane, clean-up procedures and analysis by GC/TEA. Extracts from both the buffer solution and the filter were cleaned up and analyzed individually. The clean-up procedure included column chromatography on basic alumina and the removal of nicotine. *N*-Nitrosodibenzylamine [NDBenzA] which had suitable chromatographic properties was used as internal standard. *N'*-Nitrosopentylpicolylamine [NPePicA], a newly synthesized nitrosamine with physicochemical properties similar to the TSNA, could also be used as an internal standard. The recoveries for the buffer solution were as follows: NDBenzA 89%, NPePicA 83%, NNN 85%, NNK 88%, NAB/NAT 85%. For the TSNA trapped on the filter the following recoveries were determined: NDBenzA 81%, NPePicA 75%, NNN 77%, NNK 78%, NAB/NAT 75%.
Parameters influencing the TSNA values in the mainstream smoke were investigated. Artifact formation of TSNA during mainstream smoke collection did not occur under standard conditions. TSNA values were greatly influenced by the puff volume, they increased with increasing puff volume.
Reproducible and reliable results can be achieved in a relatively short time with the method reported.

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ZUSAMMENFASSUNG

Die in der Literatur beschriebene Methode zur Bestimmung tabakspezifischer Nitrosamine [TSNA], *N'*-Nitrosonornicotin [NNN], 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanon [NNK], *N'*-Nitrosoanabasin [NAB] und *N'*-Nitrosoanatabin [NAT], im Hauptstromrauch wurde verbessert. Das hier vorgestellte Verfahren umfaßt das Auffangen des Hauptstromrauches in einer Kombination aus Flüssigkeitsfalle mit Pufferlösung und einem Cambridge-Filter, Extraktion der TSNA aus der Pufferlösung und dem Filter mit Dichlormethan, Aufarbeitungsschritten und Analyse mittels GC/TEA. Die Extrakte von Pufferlösung und Filter wurden einzeln aufgereinigt und analysiert. Die Anreicherung der TSNA erfolgte mittels Säulenchromatographie über basisches Aluminiumoxid und Entfernung von Nicotin. *N*-Nitrosodibenzylamin [NDBenzA], das geeignete chromatographische Eigenschaften aufweist, wurde als interner Standard benutzt. *N'*-Nitrosopentylpicolylamin [NPePicA], ein neu synthetisiertes Nitrosamin mit physikochemischen Eigenschaften, welche mit denen der TSNA vergleichbar sind, konnte auch als in-

terner Standard eingesetzt werden. Die Wiederfindungsraten der TSNA in der Pufferlösung waren: NDBenzA 89 %, NPePicA 83 %, NNN 85 %, NNK 88 %, NAB/NAT 85 %. Für den Filterextrakt wurden folgende Wiederfindungsraten ermittelt: NDBenzA 81 %, NPePicA 75 %, NNN 77 %, NNK 78 %, NAB/NAT 75 %.
Parameter, die die TSNA-Werte im Hauptstromrauch beeinflussen, wurden überprüft. Artefaktbildung von TSNA während des Auffangens des Hauptstromrauches konnte unter Standardbedingungen nicht beobachtet werden. Die TSNA-Werte wurden stark vom Zugvolumen beeinflußt, sie nahmen mit zunehmendem Zugvolumen zu.
Mit der vorgestellten Methode können reproduzierbare und verläßliche Ergebnisse mit geringem Zeitaufwand erreicht werden.

RESUME

Des améliorations ont été apportées à la méthode utilisée habituellement pour la détermination des teneurs en nitrosamines spécifiques du tabac [TSNA], *N'*-nitrosonornicotine [NNN], 4-(méthylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], *N'*-nitrosoanabasine [NAB] et *N'*-nitrosoanatabine [NAT], dans le courant principal de la fumée. Dans le procédé présenté ici, le courant principal a été retenu dans un dispositif combinant un piège à liquide avec solution tampon et un filtre Cambridge, les TSNA étant extraites de la solution tampon et du filtre par le dichlorométhane. Les produits extraits respectivement de la solution tampon et du filtre ont été purifiés et analysés séparément par GC/TEA. Le traitement des TSNA a compris chromatographie sur colonne (alumine basique) et élimination de la nicotine. C'est la *N*-nitrosodibenzylamine [NDBenzA] qui a été utilisée comme étalon interne en raison de ses propriétés chromatographiques bien adaptées. Il a été aussi possible de prendre la *N'*-nitrosopentylpicolylamine [NPePicA], une nouvelle nitrosamine de synthèse dont les propriétés physico-chimiques sont comparables à celles des TSNA. Les taux de récupération des TSNA dans la solution tampon ont été les suivants: NDBenzA 89 %, NPePicA 83 %, NNN 85 %, NNK 88 %, NAB/NAT 85 %. En ce qui concerne le produit extrait du filtre, les taux ont été: NDBenzA 81 %, NPePicA 75 %, NNN 77 %, NNK 78 %, NAB/NAT 75 %.
Les paramètres influant sur les quantités de TSNA contenues dans le courant principal ont été examinés également. La formation d'artefact de TSNA pendant le piégeage du courant principal n'a pu être observée dans les conditions expérimentales standard. Il a été constaté que le volume des bouffées avait une forte incidence sur les quantités de TSNA qui deviennent plus élevées lorsqu'il augmente.
La méthode présentée permet d'obtenir des résultats reproductibles et fiables en un temps relativement réduit.

INTRODUCTION

Tobacco-specific nitrosamines [TSNA]* show the highest concentrations of any group of carcinogens in the mainstream smoke of cigarettes (1) and the highest concentrations of carcinogenic nitrosamines reported in other consumer products (2). Two of the known TSNA, *N'*-nitrosonornicotine [NNN] and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], are powerful carcinogens which induce cancer in the nasal cavity, oesophagus, lung and liver of laboratory animals (3).
For a research program on TSNA in the mainstream smoke of a representative number of European cigarettes (4) a practicable method for their determination was needed. Other authors (5—9) have previously reported on TSNA determinations in the mainstream smoke of cigarettes. They all used the sampling and rather time-consuming clean-up procedure of HECHT et al. (5). All authors with the exception of ARRENDALE et al. and KLUS and KUHN (9, 6) used ^{14}C-NNN as the internal standard. It was important for us to use an internal standard which can be detected by the same method as the TSNA. It should have a recovery rate and chromatographic properties similar to those of the TSNA. Another important point was to have a less time-consuming clean-up procedure. The method of HECHT et al. was modified for our purposes.
To test the reliability and reproducibility of the results, parameters influencing the TSNA values such as artifact formation, puff volume and puff profile had to be investigated.

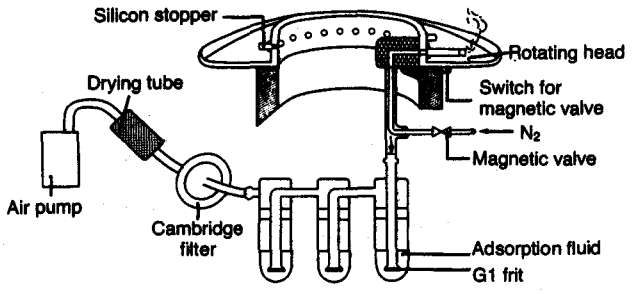
MATERIALS AND METHODS

Apparatus

For the analysis of the mainstream smoke of cigarettes a modified 30-port smoking machine with a rotating head (Type Hamburg II, Heinr. Borgwaldt, Hamburg, Federal Republic of Germany) was used. According to HOFFMANN et al. (10, 11) cigarettes were only put in every second port. Every other port was connected to a nitrogen source. In between the smoking intervals nitrogen was automatically flushed through the smoking head by means of a magnetic valve device to displace the smoke remaining within the connection to the wash bottles. This was done to prevent artifactual TSNA formation. The mainstream smoke was passed through 3 gas wash-bottles containing a total of 250 ml citrate-phosphate buffer (pH 4.5) and 20 mM ascorbic acid (5),

* The abbreviations used and the chemical substance prime names according to CHEMICAL ABSTRACTS in square brackets are: TSNA for tobacco-specific nitrosamines, NNN for *N'*-nitrosonornicotine [3-(1-nitroso-2-pyrrolidinyl)-pyridine], NNK for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, NAB for *N'*-nitrosoanabasine [3-(1-nitroso-2-piperidinyl)-pyridine], NAT for *N'*-nitrosoanatabine [1,2,3,6-tetrahydro-1-nitroso-2,3'-bipyridine], NDBenzA for *N*-nitrosodibenzylamine, NPePicA for *N'*-nitrosopentylpicolylamine, TEA for thermal energy analyzer, GC for gas chromatography.

Figure 1.
Smoking device for the analysis of tobacco-specific nitrosamines [TSNA] in mainstream smoke.



followed by a 92 mm Cambridge filter and a continuously working pump (Figure 1). NPePicA (1 µg in 1 ml ethanol) and/or NDBenzA (1 µg in 1 ml ethanol) were added to the first wash bottle as internal standards.

For the investigations into the nitrosation potential of freshly generated mainstream smoke the following solvents were used: 2 M NaOH (6), water, cyclohexane, citrate-phosphate buffer containing ascorbic acid (5). In some experiments the order of the adsorption traps was reversed, i.e. the smoke was first passed through the Cambridge filter and then through the wash bottles.

For some comparative analyses a single-port smoking machine with a piston pump (Type RM 1/G, Heinr. Borgwaldt) and a 44 mm silicon-bound glass-fibre filter for smoke collection was used.

The determination of the puff profile was carried out using a differential pressure monitor (PD 3V, Hottinger Baldwin Messtechnik, Darmstadt, Federal Republic of Germany). The puff volume was measured with a bubble flow meter (Heinr. Borgwaldt, Hamburg). GC analyses were performed on a Hewlett-Packard Model 5880A gas chromatograph directly connected to a thermal energy analyzer (TEA 502, Thermo Electron Corporation, Waltham, Massachusetts, U.S.A.) as a detector with a modified pyrolyzer. A CTR filter (Thermo Electron Corporation) was used to trap organic material after pyrolysis.

Cigarettes

The method was developed using standard cigarettes (C20) which were generously donated by Reemtsma Cigarettenfabriken GmbH, Hamburg, Federal Republic of Germany. The cigarette can be characterized by the following parameters: length 84 mm, tobacco weight approx. 790 mg / cigarette, ventilation 16%, moisture 14.0%, total alkaloids 1.63%, nicotine 1.50%, nornicotine 0.12%, anabasine/myosmine 0.007%, anatabine 0.05%, nitrate nitrogen 0.14%, tar delivery 13.7 mg / cigarette, smoke nicotine 0.99 mg / cigarette, nitrogen oxide 0.12 mg / cigarette (12). For reference analyses a commercial American-blend filter cigarette (smoke deliveries: tar 13 mg / cigarette, nicotine 0.9 mg / cigarette) and a non-filter blend cigarette (smoke deliveries: tar 20 mg / cigarette, nicotine 1.2 mg / cigarette) purchased in the Federal Republic of Germany in 1987 were also taken.

Prior to smoking, the cigarettes were kept in a chamber of 60 ± 3% relative humidity and 22 ± 2 °C for at least 24 h according to the German standard DIN 10244 (13). For practical reasons a 24.66% NaOH solution was preferred to adjust the relative humidity instead of a saturated NaBr solution.

Smoking Conditions

For each analysis, 15 cigarettes were smoked under standard smoking conditions with 1 puff/min of 35 ml volume and 2 s duration. The non-filter cigarettes were smoked to a butt length of 23 mm; the filter cigarettes were smoked to the length of the filter plus 8 mm, and filter overwrap plus 3 mm, respectively, as required by International Standard ISO 3308 (14).

The 30-port smoking machine with the continuously working pump did not generate a bell-shaped puff profile as is required by ISO 3308. A "half bell-shaped" profile was obtained when the wash bottles and the Cambridge filter were used. A bell-shaped profile was achieved with the single-port smoking machine with piston pump.

Reagents

NNK was a generous gift from Dr. D. Hoffmann, Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York, U.S.A., and was also obtained from Chemsyn Science Laboratories, Lenexa, Kansas, U.S.A. NDBenzA, NNN and NAB were synthesized by methods reported in the literature (15—17). The purity was >99%. NPePicA was synthesized as previously reported (18); purity was 98%. Nicotine and nornicotine were purchased from Roth, Karlsruhe, Federal Republic of Germany.

All the reagents used were of analytical grade.

GC Determination of TSNA

The injection-port temperature was 260 °C and the helium carrier gas flow was 30 ml / min. GC analyses were performed on a glass column (2.4 m × 2 mm inside diameter) packed with 10% OV 17 on Chromosorb WHP (80/100 mesh). The GC oven temperature was programmed using a profile starting at 180 °C with 3°/min to 230 °C, held at 230 °C for 5 minutes. The GC column was directly connected to the heated interface (300 °C) of the TEA detector's modified pyrolyzer (500 °C). All nitrosamines with the exception of NAB and NAT were baseline separated. Results were calculated by peak area comparison with the aid of an elec-

tronic integration system (Trilab 2000, SES, Nieder-Olm, Federal Republic of Germany) using an internal standard method. NPePicA and/or NDBenzA were used as internal standards. NAB and NAT were calculated as a group.

Mainstream Smoke Analysis for TSNA

A. *30-Port Smoking Machine*

The filter and the trapping fluid were cleaned up and analyzed individually.

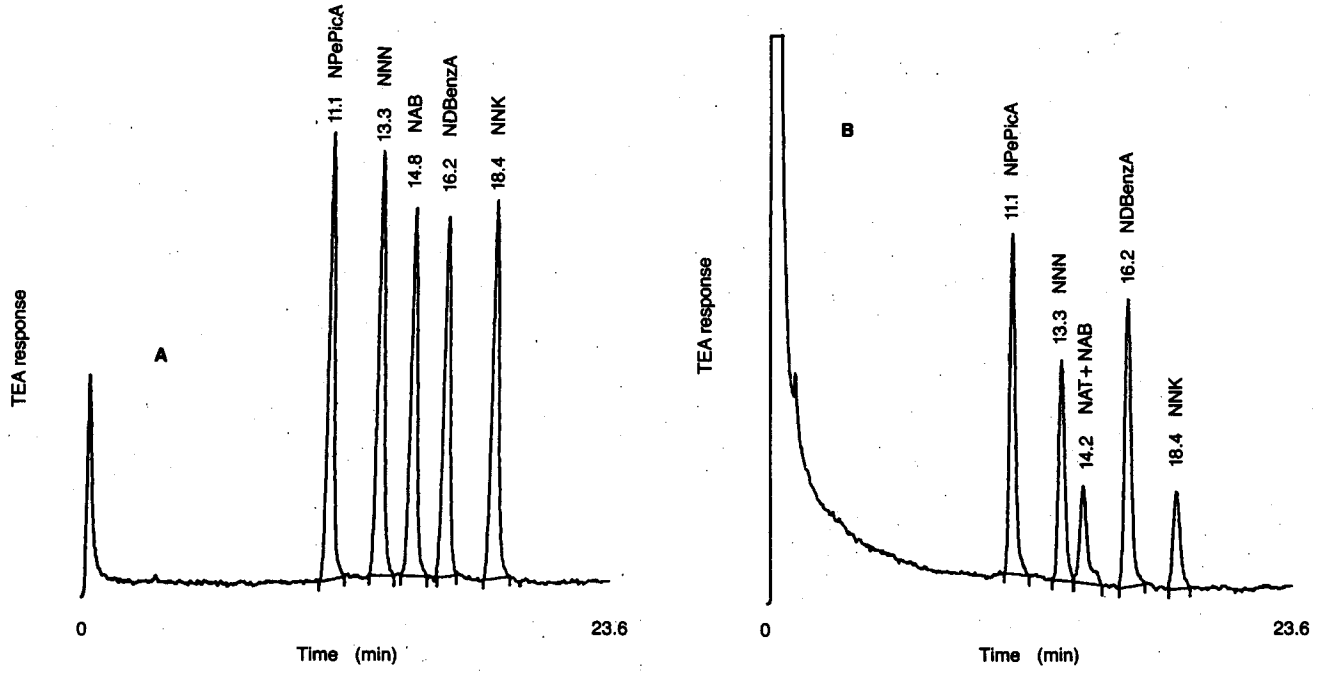
1. *Analysis of the Cambridge Filter:* The filter was extracted three times with dichloromethane to a total volume of 350 ml. NPePicA (1 µg in 1 ml ethanol) and/or NDBenzA (1 µg in 1 ml ethanol) were added to the combined dichloromethane extracts as internal standards. The extract was concentrated to 2 ml using a rotary evaporator and chromatographed on 4 g of basic alumina (activity II) on a 12 cm × 50 mm column. The TSNA and internal standards were eluted with 4 ml dichloromethane and 6 ml of a dichloromethane:methanol (10:1 (v/v)) mixture. The eluate was washed once with 7.5 ml citrate-phosphate buffer (pH 4.5) containing 20 mM ascorbic acid. This was necessary to remove nicotine from the extract. Nicotine disturbed the GC determination of the TSNA by reducing the detector response. For the re-extraction of the TSNA the buffer solution was extracted once with 2.5 ml dichloromethane and once with 2.5 ml of a dichloromethane:methanol (10:1 (v/v)) mixture. The three organic phases were combined, dried (Na_2SO_4), concentrated to 2 ml under a stream of nitrogen and analyzed by GC/TEA.

2. *Analysis of the Trapping Fluid:* The liquids from the traps were combined and extracted 3 times with 150 ml dichloromethane. The combined dichloromethane extracts were dried over Na_2SO_4, concentrated to 2 ml using a rotary evaporator and chromatographed on 4 g of basic alumina as described above. The additional washing procedure to remove nicotine was only necessary in cases where non-acidic liquids were used for smoke trapping. The eluate was concentrated to 2 ml under a nitrogen stream and analyzed by GC/TEA.

B. *Single-Port Smoking Machine*

Ten cigarettes were directly smoked in sequence onto the same 44 mm silicon-bound glass-fibre filter. The filter was extracted with dichloromethane, 2 µg NDBenzA in 2 ml ethanol were added and the further clean-up was performed as described under A1. The final volume was 4 ml.

Figure 2.
GC/TEA chromatograms using 10% OV 17 on Chromosorb WHP as stationary phase.
A: standard mixture of *N'*-nitrosopentylpicolylamine [NPePicA], *N'*-nitrosonornicotine [NNN], *N'*-nitrosoanabasine [NAB], *N*-nitrosodibenzylamine [NDBenzA] and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK] with 0.5 µg/ml for each nitrosamine;
B: extract of the buffer solution (non-filter blend cigarette).



RESULTS AND DISCUSSION

Internal Standard

The use of any internal standard for the determination of TSNA, which themselves have different physicochemical properties such as different extraction behaviour of NNN and NNK at different pH (18), always represents a compromise. Non-labelled internal standards, *N'*-nitrosopentylpicolylamine (NPePicA), which has a similar structure to the TSNA, and *N*-nitrosodibenzylamine (NDBenzA), were used. In the literature ^{14}C-NNN is described as internal standard (5—8). Our aim, however, was to determine the recovery of the internal standard using the same detection method as for the TSNA. NDBenzA and NPePicA have gas chromatographic properties similar to the TSNA. Using OV 17 as stationary phase, NPePicA was eluted before NNN. The retention time of NDBenzA was between those of NAB/NAT and NNK, as is shown in Figure 2. With the method described, the recoveries of NPePicA and NDBenzA were similar to those of the TSNA; both were therefore useful internal standards.

Clean-up Procedure

Depending on the cigarette brand and the temperature of the buffer solution, the distribution of the TSNA between the buffer solution and the Cambridge filter varied widely. Between 7% and 50% were found on the Cambridge filter. No experiment achieved complete trapping of TSNA in the buffer solution. Buffer solution and Cambridge filter were separately extracted with dichloromethane. Since two different extractions (extraction of TSNA from buffer and from Cambridge filter) were used in the method, it was necessary to add internal standard to each extract. The recovery rates were different for filter and buffer solution. An individual internal standardization compensated for these differences and enabled us to get reproducible and accurate results.

For gas chromatography in combination with chemiluminescence detection a further clean-up procedure was necessary. The extraction of the TSNA from the buffer solution and the filter was followed by column chromatography on basic alumina. Using short columns with 4 g of basic alumina and 10 ml of eluent sufficiently clean eluates were obtained. Owing to the extraction at pH 4.5, the extracts of the buffer solution were free of nicotine whereas the filter extracts contained considerable amounts of nicotine. But nicotine interfered with GC/TEA determination and had therefore to be removed. This was done after column chromatography by washing the eluate with citrate-phosphate buffer containing 20 mM ascorbic acid. This buffer solution was re-extracted with dichloromethane and dichloromethane/methanol to improve the recovery.

With sufficiently clean samples the whole clean-up procedure can be done in less than half an hour.

Table 1.
Artifact formation of NNN after addition of 15 mg nornicotine to different solvents in the traps when 15 standard cigarettes were smoked on a smoking machine.

Solvent	NNN (ng/cigarette)
Citrate-phosphate buffer + ascorbic acid without addition of nornicotine	99
Cyclohexane	259
2 M NaOH	142
Citrate-phosphate buffer + ascorbic acid	103
H_2O	130
Cambridge filter followed by wash bottles (filter was fortified with 15 mg nornicotine and the order of the adsorption traps was reversed)	204

Artifact Formation

The nitrosation potential of freshly generated mainstream cigarette smoke was determined in different solvents using tobacco alkaloids as precursor amines. The secondary amine nornicotine was added to the solvents in the wash bottles in amounts of 1 mg per cigarette smoked (i.e. 15 mg in the trapping fluid) — the same amount as the total alkaloid concentration in the mainstream smoke of the test cigarette. Depending on the trapping fluid, nitrosation of nornicotine could be observed (Table 1). In citrate-phosphate buffer with ascorbic acid no nitrosation to NNN was detectable. In water and alkaline solution an increase in NNN was observed, which amounted to about 30% and 40% respectively, whereas a more than 100% increase in NNN was detected in cyclohexane. The nornicotine content of tobacco can vary widely but normally cigarette tobacco does not contain more than 10% of the total alkaloid concentration as nornicotine (12, 19), i.e. not more than 0.1 mg per standard cigarette smoked accounted for nornicotine in the mainstream smoke. These data indicate that in water and in 2 M NaOH 3 ng / cigarette NNN and 4 ng / cigarette NNN respectively might be attributed to artifact formation. In cyclohexane 16 ng / cigarette NNN would be formed as a result of artifact formation. Thus for the standard cigarette with 99 ng of NNN per cigarette only about 16% of NNN could be attributed to artifact formation in cyclohexane. If H_2O or 2 M NaOH were used for trapping, 3% to 4% of NNN would be due to artifact formation, which is within the range of standard deviation for the method. Therefore, artifactual formation of NNN from nornicotine virtually does not play an important role in TSNA analysis.

The major tobacco alkaloid nicotine, containing a tertiary amino group, was also investigated with regard to its possible artifactual formation. Wash bottles contain-

ing cyclohexane were fortified with nicotine in amounts of 1 mg and 10 mg nicotine per cigarette smoked (i.e. 15 mg and 150 mg in the trapping fluid). No additional NNN or NNK were observed. Furthermore, no artifactual NNK formation was observed at elevated temperatures up to 60 °C.

When the order of the adsorption traps was reversed, i.e. when the smoke was first passed through the Cambridge filter and then through the gas wash-bottles, the TSNA values determined for the standard cigarettes were the same within the normal ranges of deviation as those obtained with citrate-phosphate buffer with 20 mM ascorbic acid. In this case, all the TSNA were found on the Cambridge filter.

Using the standard cigarette, artifactual formation of NNN or NNK from nicotine was not observed. Artifact formation of TSNA from secondary amines had no influence on the TSNA values in the mainstream smoke. The low nitrosation potential was not surprising since freshly generated tobacco smoke contains only nitric oxide and no nitrogen dioxide (20). But nitric oxide alone is not a nitrosating agent (21).

To exclude the probability of artifact formation under conditions which cannot be represented by the standard cigarette, it was decided to include citrate-phosphate buffer in the trapping procedure.

Criteria for Reliability

Chemiluminescence detection in combination with gas chromatography is highly specific for compounds producing NO upon pyrolysis. In all analyses only nitrosamine-related peaks were detected.

For optimal detector performance organic material eluting from the pyrolyzer has to be trapped and this was done with a filter device. Nitric oxide generated from nitrosamines in the pyrolyzer can pass through the device unaffected. In the presence of amines, however, trapping or adsorption of nitric oxide can occur which results in a decrease of the detector signal (22). When nicotine was present in the smoke extracts, the response of the TEA detector was drastically reduced. The reduction of the detector signal was the same for all the TSNA and the internal standard. To improve the sensitivity of the method and to avoid interference with amines, nicotine had to be removed in the course of analysis.

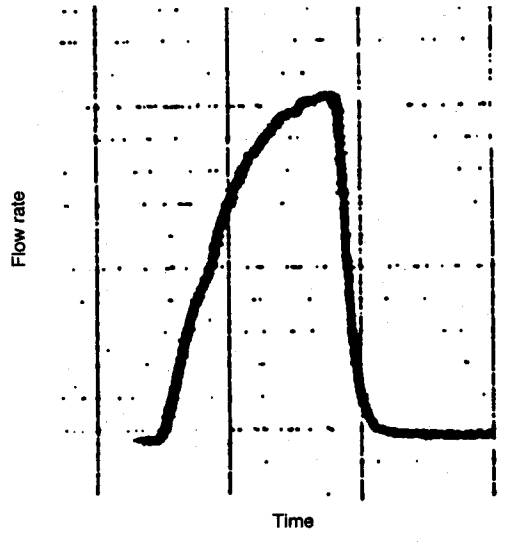
The GC parameters were optimized for NNN and NNK. Under these conditions NAB and NAT could not be separated. Optimum conditions for resolving NAB and NAT resulted in a broadening of the NNK peak and increased run times. Since our interest was mainly focused on the fast and reliable determination of NNN and NNK, the nitrosamines NAB and NAT were calculated as a group. For these reasons a 2.4 m glass column packed with 10% OV 17 on Chromosorb WHP was chosen and proved to be satisfactory especially for NNN and NNK.

The overall recovery of the method was calculated using NDBenzA as internal standard. Our aim was to utilize an internal standard which has physicochemical properties that are close to those of the TSNA. Among several pyridine-derived nitrosamines, NPePicA proved to be an ideal internal standard for TSNA analysis. To prove the suitability of NPePicA and NDBenzA as internal standards, a recovery study under smoking conditions was performed. One half of the filter was spiked before smoking and one half of the buffer solution was spiked after smoking with 1 µg of each of the following: NNN, NNK, NAB, NDBenzA and NPePicA. The other parts of filter and buffer solution were spiked with 1 µg of each internal standard. For the calculation the TSNA concentration which was originally present in the mainstream smoke was subtracted from the TSNA concentration obtained after spiking. This difference was compared to the nitrosamine concentration used for spiking and the resulting recoveries were determined (Table 2). For the buffer solution the recoveries were as follows: NDBenzA 89%, NNN 85%, NNK 88%, NAB/NAT 85%, NPePicA 83%. For the

Table 2.
Recovery rates of TSNA, NDBenzA and NPePicA (1 µg of each nitrosamine was added to the buffer solution and the Cambridge filter).

	Percentage recovery rates	
	buffer solution	Cambridge filter
NDBenzA	89	81
NPePicA	83	75
NNN	85	77
NNK	88	78
NAB/NAT	85	75

Figure 3.
Diagrammatic representation of the "half bell-shaped" puff profile.



filter the following recovery rates were determined: NDBenzA 81%, NNN 77%, NNK 78%, NAB/NAT 75%, NPePicA 75%. These results show that the recovery rates of the individual TSNA are comparable with the recoveries of NPePicA and NDBenzA. This allows an accurate correction for losses in the course of analysis using NDBenzA or NPePicA as internal standard. However, it was occasionally observed that NDBenzA was recovered at a much lower rate than NPePicA and the TSNA. Calculating the TSNA on NPePicA as the internal standard resulted in more accurate values. NDBenzA was suitable only if its recovery was at least 60%. Analyses with a lower recovery had to be repeated or calculated based on NPePicA.

The minimum detection limit for NNN, NNK and NAB/NAT respectively was 4 ng / cigarette with a GC/TEA detection limit of 0.15 — 0.25 ng / injection. The mean value of 5 analyses in the mainstream smoke of a filter cigarette was 69 ± 11 ng / cigarette for NNN, 42 ± 4 ng / cigarette for NNK and 68 ± 9 ng / cigarette for the sum of NAB and NAT.* Since no artifactual formation of NNK and only negligible artifactual formation of NNN was observed, the actual TSNA concentration in mainstream smoke was determined.

Considering that cigarette tobacco is not completely homogeneous the data shown account for good reproducibility and reliable results.

Influence of Puff Profile

The standard cigarette and a consumer filter cigarette were smoked with different puff profiles: bell-shaped profile and "half bell-shaped" profile (Figure 3). The other smoking parameters were held constant. The results obtained with the bell-shaped profile and the "half bell-shaped" profile were the same within the normal ranges of deviation (Table 3).

Thus the "half bell-shaped" profile did not influence the TSNA delivery in mainstream smoke.

Influence of Puff Volume

A first application of this method was to investigate the influence of the puff volume. For this purpose only two cigarette types were chosen, the standard cigarette (C20) and a non-filter German blend cigarette. Both brands were smoked with different puff volumes. The other smoking conditions were held constant. For all TSNA the mainstream smoke values increased with increasing puff volume (Figure 4). For these two cigarette types the relationship was linear. It should be pointed out that these two examples cannot be generalized. Publication of a more detailed study on parameters influencing the TSNA yield in mainstream smoke is in preparation.

* For the repeatability study a different batch of C20 cigarettes had to be used which showed a significant difference in TSNA amounts in mainstream smoke.

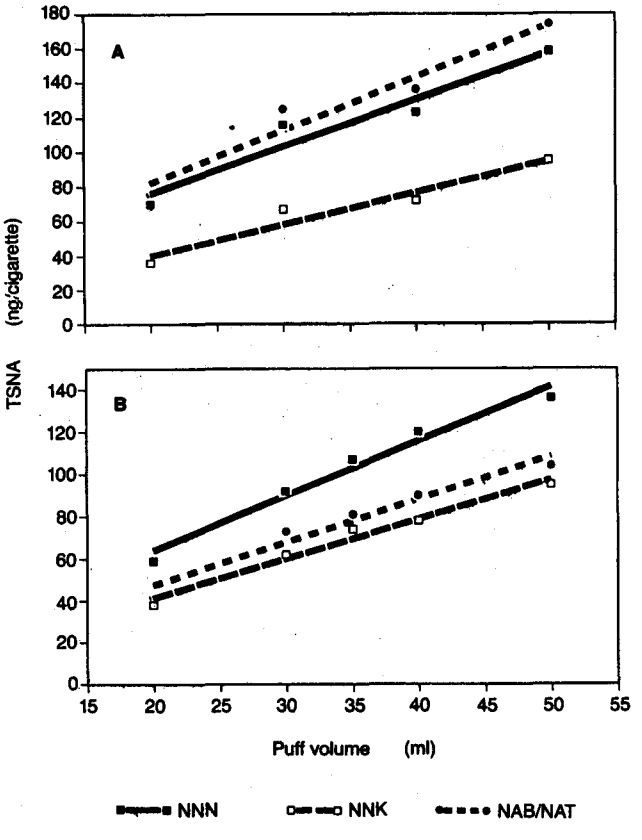
Table 3.
TSNA values of two different brands smoked with a different puff profile.

	NNN (ng/cigarette)	NNK (ng/cigarette)	NAB + NAT (ng/cigarette)
Test cigarettes			
"half bell-shaped" profile	99	56	107
bell-shaped profile	85	61	99
American-blend filter cigarettes			
"half bell-shaped" profile	179	142	259
bell-shaped profile	215	145	285

CONCLUSIONS

The method reported is suitable for determining TSNA in the mainstream smoke of cigarettes. We consider it to be an improvement over other methods reported in

Figure 4.
TSNA values as a function of puff volume (A: standard cigarette, B: non-filter blend cigarette).



the literature because NDBenzA and NPePicA are used as internal standards. The recovery rates of NDBenzA and NPePicA are determined by the same detection method as the TSNA. The method accounts for good reproducibility and high sensitivity (detection limit: 0.15 — 0.25 ng / injection, determination limit: 4 ng / cigarette for NNN, NNK and NAB/NAT respectively). Another advantage of the method lies in the less time-consuming clean-up procedure. As the entire clean-up procedure can be carried out in a shorter time than that of HECHT et al. (5), more analyses can be performed in the same time.

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