DOI:10.1021/jf103772c

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Evaluation of Two Derivatization Reagents for the Determination by LC-MS/MS of Ammonia in Cigarette Mainstream Smoke

Nicolas Mottier* and Florent Jeanneret

Philip Morris International R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland

Ammonia in cigarette mainstream smoke was quantified by LC-MS/MS after derivatization. Two different reagents, fluorescamine and dansyl chloride, were investigated, but only the latter gave stable derivatives; therefore, it was considered the most appropriate choice. Smoke samples were collected on a Cambridge filter pad followed by an impinger containing a solution of hydrochloric acid. Ammonia was then derivatized with a 18.5 mM solution of dansyl chloride in acetonitrile at 70 °C for 30 min in a vial with the internal standard, $^{15}ND_4Cl$. The resulting derivative was analyzed by LC-MS/MS detection with an atmospheric pressure chemical ionization (APCI) interface in the positive ionization mode using multiple-reaction monitoring (MRM). Good linearity was obtained in the concentration range of 0.02−1.65 μg/mL ($r^2 \geq 0.999$), and the limit of detection (LOD) was established at 0.006 μg/mL. This method has the advantage of being sensitive, efficient, and reliable and is not hindered by interferences from the sample matrix. It should thus be considered a reference method of choice for the determination of ammonia in smoke.

KEYWORDS: Ammonia; cigarette mainstream smoke; fluorescamine; dansyl chloride; LC-MS/MS

INTRODUCTION

Ammonia, one of the major constituents of cigarette smoke, results from the reduction of nitrates and the pyrolysis of amino acids (such as glycine (*1*)), proteins, and other nitrogen-containing compounds, which means it is quite sensitive to the composition of the tobacco blend (*1*).

There are no international standard methods available for the determination of mainstream smoke constituents other than tar (*2*), nicotine (*3*), carbon monoxide (*4*), and benzo[*a*]pyrene (*5*). The trapping of ammonia is commonly performed by collecting the smoke on a Cambridge filter pad followed by one or two impingers filled with an acidic solution, for example, sulfuric acid or hydrochloric acid (*6*−*15*). Electrostatic precipitation of the aerosol can also be used for collection (*16*). Ensuing analyses are performed by ion chromatography coupled to a conductivity detector (*11*, *12*, *15*, *16*). Other methodologies are determination with an ammonia electrode (*17*) or by colorimetric means (*18*, *19*). As noted in the literature, the variability obtained with these methods is relatively high, and it could result in laboratories not detecting differences between smoke yields from the standard Kentucky Reference Cigarettes 1R5F and 2R4F (*10*, *20*). Also, as noted by Huang et al. (*11*), the choice of the ion chromatographic column is crucial for ensuring optimal separation of the ammonium peak from other contaminants. Common values reported for various brands under the International Standard for Organization (ISO) smoking regimen are between 3 and 30 μg/cigarette (*7*, *8*), with typical yields for the 2R4F and 1R5F of 11.5 and 3.1 μg/cigarette, respectively (*6*, *20*, *21*). Under the more intensive Health Canada smoking regimen, characterized by a 55 mL puff volume and a puff taken every 30 s, yields can be as high as 90 μg/cigarette for commercial brands (*21*).

Precolumn derivatization techniques for amino acids, amines, and peptides are numerous and offer several advantages, notably simple procedures, increased sensitivity, and reactions that are quick, quantitative, and reproducible (*22*−*26*). Fluorescamine and dansyl chloride (DNS) have both been successfully used for the quantitation of ammonia or other amino acids in various matrices such as water (*27*−*29*), urine (*30*−*32*), air (*12*), and plant and animal tissues (*33*, *34*). Therefore, the objectives of the present study were to evaluate these two derivatization reagents with the aim of increasing reliability, robustness, selectivity, and sensitivity of the commonly used methods.

MATERIALS AND METHODS

Reagents. Ammonium chloride, dansyl chloride (1-dimethylamino-naphthalene-5-sulfonyl chloride), 1-butylamine, sulfuric acid 95−97%, hydrochloric acid 32%, formic acid, boric acid, sodium carbonate (puriss. p.a.), and sodium hydroxide were purchased from Fluka (Buchs, Switzerland). Fluorescamine (4-phenylspiro-[furan-2(3*H*),1-phthalan]-3,3′-dione) was purchased form Sigma-Aldrich (Buchs, Switzerland), labeled ammonium chloride ($^{15}ND_4Cl$) from Aldrich (Buchs, Switzerland), and HPLC-grade acetonitrile from Romil (Waterbeach, Cambridge, U.K.).

Samples. The test cigarettes, provided by the University of Kentucky, were reference cigarettes 1R5F, 2R4F, and 3R4F, which were developed for research purposes in a joint effort by the National Cancer Institute of the U.S. National Institutes of Health, the Agriculture Research Service of the U.S. Department of Agriculture, and the University of Kentucky Tobacco and Health Research Institute. The 1R5F and the 2R4F cigarettes were used to develop the method. The 3R4F cigarette is a remake of

*To whom correspondence should be addressed. Telephone: +41 (58) 242 2375; Fax: +41 (58) 242 2811; E-mail: nicolas.mottier@pmintl.com.

 Published on Web 12/13/2010

the 2R4F and intended to replace the depleted stock of 2R4F cigarettes. In general, these reference cigarettes are representative of conventional commercial cigarettes of similar total particulate matter (TPM) yield. The reference cigarettes are 84 mm in length and 25 mm in circumference, with a butt length of 35 mm. TPM yields are lower for 1R5F than for 2R4F and 3R4F (2.1 mg/cigarette, compared to 11.7 and 10.9 mg/cigarette, respectively), as are nicotine yields (0.16 mg/cigarette compared to 0.85 and 0.73 mg/cigarette, respectively). Prior to smoking, the cigarettes were conditioned at 22 ± 1 °C with a relative humidity of 60 ± 2% and an atmospheric pressure of 96 ± 10 kPa for at least 2 days (ISO standard 3402).

Sample Collection. *For the Fluorescamine Method.* Ten cigarettes per brand were smoked on a 20-port rotary Borgwaldt smoking machine equipped with a 92 mm Cambridge filter pad followed by one wash bottle containing 25 mL of a 0.05 M H_2SO_4 solution for the trapping of ammonia. Mainstream smoke was generated according to ISO guideline 3308 (35 mL puff volume and a 2 s puff duration every minute). The content of the impinger was added to a flask with the pad and shaken for 30 min on a rotary shaker at 2500 rpm. A 5 mL aliquot of the solution was filtered through a 0.45 μm Millipore membrane (Billerica, MA) into a flask before derivatization.

For the Dansyl Chloride Method. Five cigarettes per brand were smoked on a 20-port rotary Borgwaldt smoking machine equipped with a 44 mm Cambridge filter pad followed by one wash bottle containing 25 mL of a 0.005 M HCl solution for the trapping of ammonia (*11*). Mainstream smoke was generated according to ISO guideline 3308. The pads were weighed before and after each run to determine the amount of TPM. If the TPM was > 3 mg, then an additional 25 mL of acid was added to the wash bottle before extraction. Once this was determined, the content of the impinger was added to a flask with the pad and shaken for 30 min on a rotary shaker at 2500 rpm. A 5 mL aliquot of the solution was filtered into a flask before derivatization.

Derivatization Methods. *Fluorescamine Derivatization.* An 18.69 mM ammonium chloride stock solution was prepared in a 0.2 M sulfuric acid solution. Standards were prepared by appropriate dilution with 0.05 M sulfuric acid to have a NH_3-equivalent concentration range from 37 to 2198 ng/mL. A 2.7 mM 1-butylamine stock solution was made in a 0.2 M sulfuric acid solution and used as internal standard. The working solution was made by dilution of 1 mL of the stock solution into a 10 mL volumetric flask with 0.05 M sulfuric acid. A borate buffer solution was made by dissolving 1.24 g of boric acid in a 100 mL volumetric flask with a 0.1 M sodium hydroxide solution. The fluorescamine derivatization solution was prepared by dissolving 18.75 mg of fluorescamine in a 25 mL volumetric flask with acetone. Derivatization was done by pipetting the following volumes in a 2 mL vial: 370 μL of the calibration solution or sample, 30 μL of the internal standard (ISTD) 1-butylamine solution, 400 μL of the borate buffer, 400 μL of 0.1 M sodium hydroxide, and 400 μL of the fluorescamine solution.

Dansyl Chloride Derivatization. An 18.69 mM ammonium chloride stock solution was prepared in a 0.005 M hydrochloric acid solution. The working calibration solutions were made by appropriate dilution with 0.005 M hydrochloric acid to have a concentration range from 21 to 1650 ng/mL. A carbonate solution was made by dissolving 200 mg of sodium carbonate in a 100 mL volumetric flask with deionized water. The derivatization solution was prepared by dissolving 50 mg of dansyl chloride in a 100 mL volumetric flask with acetonitrile. The internal standard solution was made by measuring 30 mg of $^{15}ND_4Cl$ into a 50 mL volumetric flask and diluting to volume with 0.005 M HCl. Derivatization was done by pipetting, in the following order in a 2 mL vial, 370 μL of the calibration solution or sample, 30 μL of the ISTD solution, 17 μL of carbonate solution, and 1 mL of the dansyl chloride solution. The vials were vortexed for 20 s, and then the reactions was left to proceed for 30 min in an oven at 70 °C. They were subsequently cooled to room temperature before being injected. Derivatized standards and samples were stable for up to 3 days.

Instrumentation and Analytical Conditions. Both derivatives were analyzed on a Thermo-Fisher Scientific TSQ Quantum Discovery LC-MS/MS equipped with a CTC Analytics autosampler and a Rheos HPLC pump. The HPLC column used was an Agilent Zorbax RP C-18 (150 × 3.0 mm, 3.5 μm particles) equipped with a Phenomenex C-18, 5 μm, guard column.

Fluorescamine LC-MS/MS Analytical Conditions. HPLC mobile phase A was an acetate buffer, and mobile phase B was acetonitrile. The buffer consisted of 2 mM ammonium acetate in deionized water, adjusted to pH 3 with acetic acid. Both water and acetonitrile were filtered through a 0.45 μm Millipore membrane (Billerica, MA). Injection volume was 10 μL, and the flow rate was set at 500 μL/min. The initial conditions were 60:40 acetate buffer/acetonitrile (v/v). The run was programmed for a gradient elution with a linear ramp to 30:70 from 0 to 6 min. The column was then re-equilibrated at 60:40. The instrument was equipped with an ESI ionization source set in the positive mode. Detection was performed by multiple-reaction monitoring (MRM). The MS/MS parameters were set as follows: spray voltage at 4.0 kV, transfer capillary temperature at 350 °C, sheath gas at 20 AU, auxiliary gas at 10 AU, Q_2 collision gas (argon) at 1.5 mTorr, and collision energy at 10 V. The transitions monitored were from m/z 296.0 to 278.0 for the ammonia derivative and from m/z 352.0 to 334.0 for the internal standard. Two segments were created, one for the ammonia derivative and one for the internal standard. The retention time was 2.90 min for NH_3 and 5.3 min for the internal standard.

Dansyl Chloride LC-MS/MS Analytical Conditions. The HPLC mobile phase consisted of a 70:30 (v/v) mixture of acetonitrile with deionized water, both solvents filtered through a 0.45 μm Millipore membrane. The run was isocratic at a flow rate of 500 μL/min. Injection volume was 10 μL. The instrument was equipped with an APCI ionization source set in the positive mode. Detection was performed by MRM. The MS/MS parameters were set as follows: discharge current at 4.0 kV, transfer capillary temperature at 340 °C, sheath gas at 40 AU, and auxiliary gas at 5 AU. The collision energy was set at 20 V and the Q2 collision gas (argon) at 1.5 mTorr. The transitions monitored were m/z 251.0 → 236.0 for NH_3 and m/z 254.0 → 239.0 for the internal standard. The retention time was 2.3 min for both derivatives.

RESULTS AND DISCUSSION

Derivatization with Fluorescamine. The first derivatization agent to be evaluated was fluorescamine. It is a heterocyclic dione that reacts with primary amines, including peptides, amino acids, and proteins, to form fluorescent products, that is, pyrrolinones (*24*, *32*, *35*−*43*). At the optimal pH of 8−9 and when the reagent is dissolved in a nonhydroxylic solvent, such as acetone or acetonitrile, the half-time of the reaction is a few milliseconds at room temperature (*25*, *37*).

The derivatization conditions were adapted from the literature (*24*, *25*, *29*, *37*, *43*), notably by adjusting the buffering conditions to the smoke matrix. Cigarette mainstream smoke samples were collected with a combination of a Cambridge filter pad and an impinger filled with 25 mL of an aqueous sulfuric acid solution, on the basis of a modified version of the Health Canada method (*13*). Determination was performed by LC-MS/MS using an electrospray ionization (ESI) source in the positive mode. The LC method had to be optimized to separate the ammonia derivative peak from matrix interferences. Optimal resolution was reached by setting the flow rate at 500 μL/min and starting the run with a 60:40 acetate buffer/acetonitrile followed by a 6 min gradient to reach 30:70. The derivative peak was detected at a retention time of 2.9 min and that of the internal standard at 5.3 min.

Linearity was established over the concentration range of 0.037−2.224 μg/mL with a correlation coefficient of $r^2 \geq 0.999$. The limit of quantitation (LOQ) was calculated as 0.033 μg/mL (0.065 μg/cigarette) and the limit of detection (LOD) as 0.009 μg/mL (0.019 μg/cigarette), on the basis of the variance of the calculated amount for five replicates for the standard with the lowest concentration. Analysis of 1R5F and 2R4F smoke extracts showed yields of 3.25 μg/cigarette (standard deviation (SD), 0.19; relative standard deviation (RSD), 5.8%; $n = 5$) and 10.67 μg/cigarette (SD, 0.20; RSD, 1.8%; $n = 5$), respectively, which is in line with the literature (*36*).

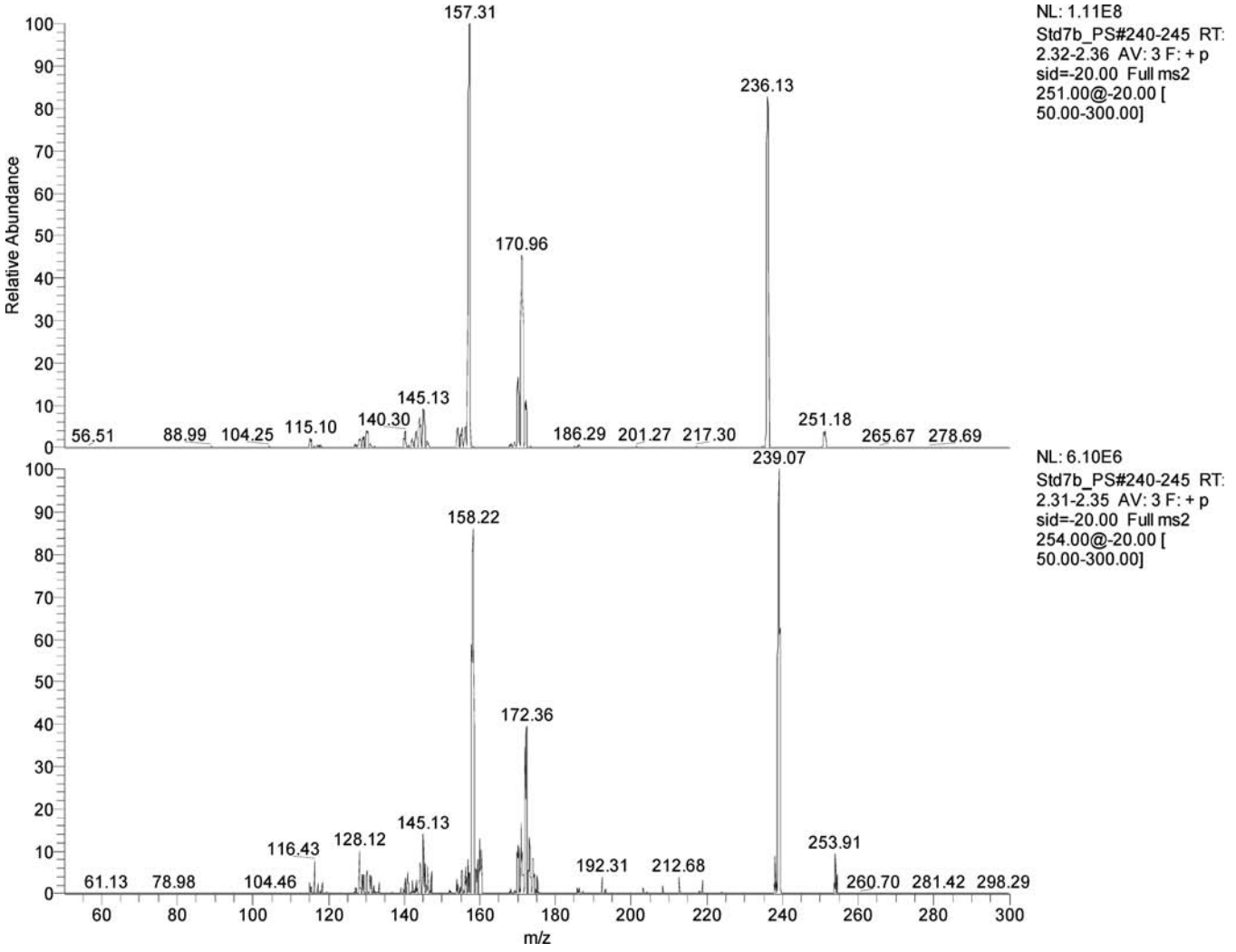

Figure 1. Product scan mass spectra of the ammonia−DNS derivative (top) and the internal standard (bottom) of a 3R4F smoke extract.

Although these results were found to be promising, standards were found to be stable for <24 h when stored at −4 °C. Repeated injections of the standards showed a loss of linearity of the calibration curve, which was illustrated by the correlation coefficient r^2 dropping over 3 days (first day, 0.9999; second day, 0.9990; third day, 0.9897). This could be due to the fluorescamine derivatives that are hydrolyzed to form nonfluorogenic compounds (*43*). This limited stability meant that the method was unsuitable for our purposes; therefore, we assessed the second derivatization agent, dansyl chloride.

Derivatization with Dansyl Chloride. Dansyl chloride is widely used in protein sequencing and qualitative amino acid analysis as a derivatizing agent that reacts with primary amino groups to form stable blue-green fluorescent sulfonamide adducts (*27*, *28*, *33*, *40*, *44*−*47*).

As with fluorescamine, the derivatization conditions were modified from the literature (*26*, *44*). Because the reaction is pH-dependent, the concentration and volume of the carbonate solution had to be adapted to the matrix. Using smoke samples from 1R5F and 2R4F, the best conditions resulting in the highest peaks were found when a volume between 15 and 20 μL of a 2 mg/mL carbonate solution was added. Seventeen microliters was then chosen as the best volume. The reaction time was also verified in the range of 5−45 min, and a maximum response was obtained after 30 min. The stability of the derivatized standards and samples was established at 3 days at −4 °C with the correlation coefficient of the calibration curve remaining constant (first day, 0.9997; second day, 0.9997; third day, 0.9998), which was deemed sufficient for our purposes.

The product scan mass spectra of the DNS derivatives for ammonia and the labeled internal standard are shown in **Figure 1**. The ions at *m*/*z* 236 and 239, corresponding to the loss of a methyl group, were used for the MRM transitions.

Both ESI and APCI probes were evaluated for the ionization of the derivatives. Although the ESI source gave the highest peak intensities, it showed nonlinear responses in the concentration range required. This was not the case with the APCI source, which was thus selected.

Even though competing reactions can take place between the derivatization agent, water, and amino acids when using the dansyl chloride reaction (*44*), the chromatograms of smoke samples showed no major interferences.

Comparisons between the two derivatization methods showed that lower yields were obtained by dansylation with the 2R4F samples but not with the 1R5F samples. Because the TPM yield of the 2R4F is > 5-fold that of the 1R5F (11.7 and 2.1 mg/cigarette, respectively), it was suspected that the pad was not sufficiently extracted with the 25 mL of acid. To verify this, two smoking runs were performed: one with 10 2R4F cigarettes and the second with 5. The setup was the same for both runs: the smoke was trapped on a 44 mm pad and an impinger connected behind it filled with 50 mL of 0.005 M hydrochloric acid. After collection, the acid

Table 1. Calibration Curve Parameters

standard level	concn (μg/mL)	replicates	av calcd amount (μg/mL)	SD (μg/mL)	RSD (%)	av residuals (μg/mL)
1	0.021	5	0.020	0.002	8.4	−0.0004
2	0.042	5	0.041	0.001	2.6	−0.0010
3	0.083	5	0.083	0.001	1.3	−0.0005
4	0.166	5	0.169	0.003	2.0	0.0025
5	0.333	5	0.346	0.005	1.5	0.0136
6	0.832	5	0.832	0.008	0.9	−0.0002
7	1.664	5	1.650	0.034	2.1	−0.0140

Table 2. Recovery Results

	sample A				sample B			
added (μg)	0.32	0.64	0.96	1.59	0.32	0.64	0.96	1.59
recovery (%)	102	108	109	98	106	105	106	99
SD (μg)	1.3	5.7	1.1	1.0	2.0	0.8	0.4	0.8
RSD (%)	1.2	5.2	1.0	1.0	1.9	0.7	0.3	0.8

from the impinger was used to extract the pad. The result showed that 2.4 μg/cigarette more ammonia was collected with 5 cigarettes than with 10 cigarettes (10.2 μg/cigarette compared to 7.8 μg/cigarette), hence supporting the supposition because these values were in line with the fluorescamine results and those published. Following this observation, the postcollection method was modified in the following fashion: after the smoking run, the pad was weighed, and if the TPM collected was < 3 mg/cigarette, the extraction was carried out with the content of the one impinger (25 mL); if it was > 3 mg/cigarette, an extra 25 mL of fresh acid was added.

Analytical Performance of the Dansyl Chloride Method. A 7-point calibration curve with a concentration range of 0.02−1.65 μg/mL was established with a correlation coefficient of $r^2 \geq 0.999$ using a $1/x$ weighting factor (**Table 1**). The average residual in micrograms per milliliter was established as the difference between the calculated concentration of the standard and the theoretical concentration of the standard. The LOD was established as 0.005 μg/mL (0.02 μg/cigarette with a 25 mL dilution) and the LOQ as 0.017 μg/mL (0.08 μg/cigarette). LOD and LOQ were calculated, respectively, as 3 and 10 times the square root of the variance for the lowest concentration standard.

Instrument and derivatization repeatability was checked with smoke extracts from 1R5F and 3R4F. Ten injections of each extract showed that the SD obtained was 0.03 μg/cigarette (RSD = 0.7%) for 1R5F and 0.06 μg/cigarette (RSD = 0.5%) for 3R4F. To test the derivatization procedure, the smoke extracts were derivatized 10 times each. The average ammonia yield was 3.6 μg/cigarette (SD = 0.07, RSD = 2.0%) for 1R5F and 11.3 μg/cigarette (SD = 0.15, RSD = 1.3%) for 3R4F.

Recovery of the method was performed by spiking, in triplicate, two different smoke extracts of commercial brands with four levels of a solution of ammonium chloride in 0.005 M hydrochloric acid. The results showed a recovery between 98 and 109% (**Table 2**).

An interday repeatability test was performed with 1R5F and 3R4F and two commercial brands by smoking five cigarettes of each type on three different days. Four replicates were performed for each cigarette type. The results are given in **Table 3**. The confidence interval (CI) at 95% was calculated with the formula

$$CI_{95} = \frac{t_{(n-1)}SD}{\sqrt{n}}$$

where SD is the standard deviation, n is the number of replicates (4 in this case), and $t(n-1)$ is the t value of Student's t distribution as a function of the probability (95%) and the degree of freedom ($n - 1$).

Table 3. Interday Repeatability Test Results (All Values in Micrograms per Cigarette)

	day 1		day 2		day 3	
sample	av	CI at 95%	av	CI at 95%	av	CI at 95%
1R5F	3.4	0.2	3.7	0.3	3.4	0.4
3R4F	10.9	1.4	11.5	0.6	11.0	1.0
brand A	8.5	0.4	8.9	0.6	8.4	1.0
brand B	13.4	1.0	14.0	1.1	14.3	1.0

Table 4. Interday Repeatability Test Results for the Second Laboratory (All Values in Micrograms per Cigarette)

	day 1		day 2		day 3	
sample	av	CI at 95%	av	CI at 95%	av	CI at 95%
3R4F	10.5	0.8	9.7	0.4	10.5	0.7
brand A	8.5	0.6	8.1	0.2	8.6	0.5
brand B	13.6	0.5	14.0	0.9	13.5	1.0
brand C	2.6	0.3	2.2	0.2	3.0	0.8

The variations between different instruments were evaluated by comparing in-house results (**Table 3**) with those from another laboratory using a Thermo-Fisher Scientific instrument (Accela High Speed LC coupled to an TSQ Quantum Access MAX Triple Stage) and an Agilent Zorbax Eclipse XDB-C18 (150 mm × 3.0 mm, 3.5 μm) column equipped with a Phenomenex C-18, 5 μm, guard column. That laboratory tested 3R4F and three commercial cigarettes (one in addition to the two tested in our laboratories). Four replicates per cigarette type were also analyzed. The results are given in **Table 4**. The values obtained by the two laboratories for 3R4F, brand A, and brand B (**Tables 3** and **4**) are comparable and also in accordance with published values (*6*, *9*, *20*). The repeatability obtained was good and allows for the distinction between the two types of reference cigarettes (1R5F and 2R4F/3R4F), which has not always been possible using other methods (*10*, *20*).

In summary, a new, simple, and robust method for the determination of ammonia in cigarette mainstream smoke based on a precolumn derivatization with dansyl chloride followed by LC-MS/MS is presented. The first investigations with fluorescamine were promising, but the lack of stability of the derivative was not suitable for routine analysis and was thus abandoned. This was not, however, the case with dansyl chloride: improved stability and excellent results with the samples tested show that this method can be used routinely for the determination of ammonia in cigarette mainstream smoke. A 3-day interday test showed excellent repeatability for each of the five cigarette types tested. It is an improvement over previous methods as it is more selective and reliable. This method should thus be considered as an alternative of choice for the determination of ammonia in cigarette mainstream smoke.

ACKNOWLEDGMENT

We thank Lynda Conroy, Birgit Gerstenberg, and Joerg Diekmann for their critical review of the manuscript and Chantal

Dell'Aquila from OTS for providing the data from the second laboratory.

LITERATURE CITED

(1) Davis, D. L.; Nielsen, M. T. *Tobacco: Production, Chemistry and Technology*; Blackwell: Oxford, U.K., 1999.

(2) ISO 4387:2000. Cigarettes − Determination of Total and Nicotine-Free Dry Matter Using a Routine Analytical Smoking Machine; International Organization for Standardization, Geneva, Switzerland, 2000.

(3) ISO 10315:2000. Cigarettes − Determination of Nicotine in Smoke Condensates − Gas-Chromatographic Method; International Organization for Standardization, Geneva, Switzerland, 2000.

(4) ISO 8454:2007. Cigarettes − Determination of Carbon Monoxide in the Vapour Phase of Cigarette Smoke − NDIR Method; International Organization for Standardization, Geneva, Switzerland, 2007.

(5) ISO 22634:2008. Cigarettes − Determination of Benzo[*a*]pyrene in Cigarette Mainstream Smoke − Method Using Gas Chromatography/Mass Spectrometry; International Organization for Standardization, Geneva, Switzerland, 2008.

(6) Chen, P. X.; Moldoveanu, S. C. Mainstream smoke chemical analyses for 2R4F Kentucky reference cigarette. *Beitr. Tabakforsch. Int.* **2003**, *20* (7), 448−458.

(7) Counts, M. E.; Hsu, F. S.; Laffoon, S. W.; Dwyer, R. W.; Cox, R. H. Mainstream smoke constituent yields and predicting relationships from a worldwide market sample of cigarette brands: ISO smoking conditions. *Regul. Toxicol. Pharmacol.* **2004**, *39* (2), 111−134.

(8) Hyodo, T.; Maruta, Y.; Itaya, H.; Mikita, A.; Kodera, T.; Meger, M. Evaluation of functional relationships for predicting mainstream smoke constituent machine yields for conventional cigarettes from the Japanese market. *Regul. Toxicol. Pharmacol.* **2007**, *48* (2), 194−224.

(9) Counts, M. E.; Hsu, F. S.; Tewes, F. J. Development of a commercial cigarette "market map" comparison methodology for evaluating new or non-conventional cigarettes. *Regul. Toxicol. Pharmacol.* **2006**, *46* (3), 225−242.

(10) Purkis, S. W.; Hill, C. A.; Bailey, I. A. Current measurement reliability of selected smoke analytes. *Beitr. Tabakforsch. Int.* **2003**, *20* (5), 314−324.

(11) Huang, C. B.; Bassfield, R.; Dabney, B.; Hsu, F. Determination of total ammonia in mainstream smoke. *Beitr. Tabakforsch. Int.* **2003**, *20* (6), 389−393.

(12) Risner, C. H.; Conner, J. M. Collection of ammonia in indoor air by means of a weak cation exchange cartridge. *Environ. Toxicol. Chem.* **1991**, *10* (11), 1417−1423.

(13) Health Canada. Determination of ammonia in mainstream tobacco smoke − Method T101, **1999**,

(14) Brunnemann, K. D.; Hoffmann, D. Chemical studies on tobacco smoke. XXXIV. Gas-chromatographic determination of ammonia in cigarette and cigar smoke. *J. Chromatogr. Sci.* **1975**, *13* (4), 159−63.

(15) Baker, G.; Cox, R. H.; Grantham, P. F.; Vilcins, G. Optical Spectroscopy of Tobacco and Smoke Total Ammonia in Cigarette Smoke; 1987; http://tobaccodocuments.org/pm/2023954021-4068.html.

(16) Nanni, E. J.; Lovette, M. E.; Hicks, R. D.; Fowler, K. W.; Borgerding, M. F. Separation and quantitation of monovalent anionic and cationic species in mainstream cigarette smoke aerosols by high-performance ion chromatography. *J. Chromatogr. Sci.* **1990**, *28* (8), 432−436.

(17) Sloan, C. H.; Morie, G. P. Determination of ammonia in tobacco smoke with an ammonia electrode. *Anal. Chim. Acta* **1974**, *69*, 243.

(18) Harrel, T. G.; Rush, K. L.; Sensabaugh, A. A., Jr. Colorimetric method for the determination of ammonia in tobacco smoke. *Tob. Sci.* **1975**, *19*, 145−147.

(19) Swauger, J. E.; Steichen, T. J.; Murphy, P. A.; Kinsler, S. An analysis of the mainstream smoke chemistry of samples of the U.S. cigarette market acquired between 1995 and 2000. *Regul. Toxicol. Pharmacol.* **2002**, *35* (2 I), 142−156.

(20) Intorp, M.; Purkis, S.; Whittaker, M.; Wright, W. Determination of "Hoffmann analytes" in cigarette mainstream smoke. The Coresta 2006 Joint experiment. *Beitr. Tabakforsch. Int.* **2009**, *23* (4), 161−202.

(21) Counts, M. E.; Morton, M. J.; Laffoon, S. W.; Cox, R. H.; Lipowicz, P. J. Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. *Regul. Toxicol. Pharmacol.* **2005**, *41* (3), 185−227.

(22) Szokan, G. Pre-column derivatization in HPLC of amino acids and peptides. *J. Liq. Chromatogr.* **1982**, *5* (8), 1493−1498.

(23) Sahasrabuddhey, B.; Jain, A.; Verma, K. K. Determination of ammonia and aliphatic amines in environmental aqueous samples utilizing pre-column derivatization to their phenylthioureas and high performance liquid chromatography. *Analyst* **1999**, *124* (7), 1017−1021.

(24) Udenfriend, S.; Stein, S.; Bohlen, P.; Dairman, W.; Leimgruber, W.; Weigele, M. Fluorescamine: a reagent for assay of amino acids, peptides, proteins, and primary amines in the picomole range. *Science* **1972**, *178* (63), 871−872.

(25) Weigele, M. Novel reagent for the fluorometric assay of primary amines. *J. Am. Chem. Soc.* **1972**, *94* (16), 5927−5928.

(26) Buteau, C.; Duitschaever, C. L.; Ashton, G. C. High-performance liquid chromatographic detection and quantitation of amines in must and wine. *J. Chromatogr., A* **1984**, *284* (1), 201−210.

(27) Meseguer Lloret, S.; Molins Legua, C.; Verdú Andrés, J.; Campins Falcó, P. Sensitive determination of aliphatic amines in water by high-performance liquid chromatography with chemiluminescence detection. *J. Chromatogr., A* **2004**, *1035* (1), 75−82.

(28) Meseguer-Lloret, S.; Molins-Legua, C.; Campíns-Falcó, P. Selective determination of ammonium in water based on HPLC and chemiluminescence detection. *Anal. Chim. Acta* **2005**, *536* (1−2), 121−127.

(29) Mizobuchi, M.; Tamase, K.; Kitada, Y.; Sasaki, M.; Tanigawa, K. Liquid chromatographic determination of ammonium in water. *Anal. Chem.* **1984**, *56* (3), 603−605.

(30) Mizobuchi, M.; Tamase, K.; Kitada, Y. High-performance liquid chromatographic analysis of ammonium in human urine. *Anal. Biochem.* **1984**, *137* (1), 88−92.

(31) Mou, S.; Ding, X.; Liu, Y. Separation methods for taurine analysis in biological samples. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2002**, *781* (1−2), 251−267.

(32) Seiler, N. Assay procedures for polyamines in urine, serum, and cerebrospinal fluid. *Clin. Chem.* **1977**, *23* (9), 1519−1526.

(33) Marce, M.; Brown, D. S.; Capell, T.; Figueras, X.; Tiburcio, A. F. Rapid high-performance liquid chromatographic method for the quantitation of polyamines as their dansyl derivatives: application to plant and animal tissues. *J. Chromatogr., B* **1995**, *666* (2), 329−335.

(34) Parris, N. An improved fluorometric method for the determination of ammonia and volatile amines in meat tissue by high-performance liquid chromatography. *J. Agric. Food Chem.* **1984**, *32* (4), 829−831.

(35) Berthier, L.; Marchal, R.; Maujean, A. Comparison of pectolytic and proteolytic activities of six clarifying commercial preparations. Incidence of secondary enzymatic activities on proteins in champagne musts. *J. Int. Sci. Vigne Vin* **1996**, *30* (2), 103−111.

(36) Chiacchierini, E.; Restuccia, D.; Vinci, G. Evaluation of two different extraction methods for chromatographic determination of bioactive amines in tomato products. *Talanta* **2006**, *69* (3), 548−555.

(37) De Bernardo, S.; Weigele, M.; Toome, V.; Böhlen, P.; Stein, S.; Udenfriend, S. Studies on the reaction of fluorescamine with primary amines. *Arch. Biochem. Biophys.* **1974**, *163* (1), 390−399.

(38) Hougaard, D. M. Polyamine cytochemistry: localization and possible functions of polyamines. *Int. Rev. Cytol.* **1992**, *138*, 51−88.

(39) Huff, T.; Maller, C.; Hannappel, E. HPLC and post-column derivatization with fluorescamine: isolation of actin-sequestering β-thymosins by reversed-phase HPLC. *Anal. Chim. Acta* **1997**, *352* (1−3), 239−248.

(40) Neuhoff, V. Micro-electrophoresis on polyacrylamide gels. *Mol. Biol. Biochem. Biophys.* **1973**, *14*, 85−147.

(41) Pearse, A. G. E. The common peptides and the cytochemistry of their cells of origin. *Basic Appl. Histochem.* **1980**, *24* (2), 63−73.

(42) Sabatino, G.; Chelli, M.; Brandi, A.; Papini, A. M. Analytical methods for solid phase peptide synthesis. *Curr. Org. Chem.* **2004**, *8* (4), 291−301.

(43) Stein, S.; Boehlen, P.; Udenfriend, S. Studies on the kinetics of reaction and hydrolysis of fluorescamine. *Arch. Biochem. Biophys.* **1974**, *163* (1), 400−403.

(44) Bravo, M.; Eran, H.; Zhang, F. X.; McKenna, C. E. A rapid, sensitive high-performance liquid chromatography analysis of ammonia and methylamine for nitrogenase assays. *Anal. Biochem.* **1988**, *175* (2), 482−491.

(45) Grego, B.; Hearn, M. T. W. High-performance liquid chromatography of amino acids, peptides and proteins. XXXIX. Reversed-phase high-performance liquid chromatographic analysis of Dns-amino acids: comparison of several different elution systems. *J. Chromatogr.* **1983**, *255*, 67−77.

(46) Seiler, N. Use of the dansyl reaction in biochemical analysis. *Methods Biochem. Anal.* **1970**, *18*, 259−337.

(47) Tapuhi, Y.; Schmidt, D. E.; Lindner, W.; Karger, B. L. Dansylation of amino acids for high-performance liquid chromatography analysis. *Anal. Biochem.* **1981**, *115* (1), 123−129.

Received for review September 29, 2010. Revised manuscript received November 24, 2010. Accepted November 27, 2010.