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New technique using solid-phase extraction for the analysis of aromatic amines in mainstream cigarette smoke

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Abstract

A new procedure has been developed for the quantitation of aromatic amines in mainstream cigarette smoke. Two solid-phase extraction (SPE) cleanup steps using different retention mechanisms are required to process the samples. The first step uses a cation-exchange cartridge, followed by a second step that uses a cartridge with a hydrophobic retention character. The aromatic amines eluted from the second SPE cartridge are derivatized with heptafluorobutyric anhydride and analyzed with GC–MS selected ion monitoring in the negative chemical ionization mode. This new method has several advantages over other reported techniques, being sensitive, robust, and easily automated. The detection limits ranged from 0.02 ng/cigarette for tolidine to 1.41 ng/cigarette for aniline and the recoveries were from 79 to 109%.

Keywords: Cigarette smoke; Amines, aromatic

1. Introduction

When tobacco cigarettes are burned during smoking, a cycle of combustion, pyrolysis, distillation, filtration, and nucleation takes place to form the smoke that is drawn into the mouth of the smoker [1]. This mainstream smoke is a mixture of many chemicals in the vapor and particulate phases. Primary aromatic amines, such as the simplest example aniline, can be found in the particulate phase of cigarette smoke. While there are many aromatic amines in smoke, a few have drawn the focus of researchers due to inclusion on lists from the International Agency for Research on Cancer [2,3]. Thus, well-developed analytical methods are needed to quantify the amounts and types of aromatic amines in cigarette smoke and to examine smoking exposure in relation to other sources.

The earliest reports on the determination of aromatic amines in tobacco smoke were published by Hoffmann and coworkers during the late 1960s and through the 1970s [4–6]. Using unfiltered USA 85 mm cigarettes, they found that the levels of aromatic amines ranged from approximately 120 ng/cigarette of aniline down to 5.8 ng/cigarette for 2-methyl-1-naphthylamine. With commercially available table-top GC–MS instrumentation, several groups have adopted modified versions of the Hoffmann method for aromatic amines [7–10]. The general sample preparation features of these methods include: trapping the aromatic amines from smoke on a filter pad or in an acidic solution, one or more

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liquid–liquid extractions, isolation of the amines from a Florisil column, derivatization at the amine functionality, a possible additional clean-up step using normal-phase columns or liquid–liquid extraction, and one or more evaporation/reconstitution steps.

These procedures gave acceptable results, but there were many sources of errors during the preparation, the labor was intensive and not amenable for automation, and the samples need to be processed shortly after being collected. Not only is tobacco smoke chemically complex, but many particulate phase chemicals continue to oxidize/react as samples await processing. These challenges require faster, simpler preparations to achieve excellent results in a high-throughput laboratory.

A new method for aromatic amine analysis from mainstream cigarette smoke is presented with data supporting common validation requirements. In the development of this method, the goals were to produce a relatively simple method that can be automated, to have recoveries exceeding 60%, good reproducibility, accuracy, and robustness, and to have GC–MS chromatograms without interferences. A detailed description of the experimental method and results for 1R4F and 2R4F Kentucky reference cigarettes are presented along with a comparison with other published data.

2. Experimental

2.1. Reagents and materials

The deuterium-labeled aromatic amine internal standards were obtained from CDN Isotopes (Quebec, Canada), and the unlabeled aromatic amines were obtained from Sigma (St. Louis, MO, USA), except for 3-aminobiphenyl and benzidine, which were purchased from TCI America (Portland, OR, USA). All other reagents were purchased from Sigma. A spiking solution of the labeled internal standards (I.S.) was prepared in isopropanol having a concentration of 40 ng/μL [$^2H_5$]aniline, 10 ng/μL [$^2H_9$]*o*-toluidine, and 5 ng/μL each of [$^2H_7$]2-aminonaphthalene, [$^2H_9$]4-aminobiphenyl and [$^2H_8$]benzidine. Solid-phase extraction (SPE) cartridges were obtained from Waters (Milford, MA, USA). Kentucky Reference 1R4F and 2R4F cigarettes were obtained from the Kentucky Tobacco Research & Development Center, University of Kentucky, Lexington, KY, USA. Carlton 1 mg cigarettes were commercially available in the USA. All cigarettes were conditioned at 60±2.5% relative humidity and 22±1 °C for 48 h prior to use.

2.2. Automated smoking conditions

The particulate phase smoke of 20 cigarettes was collected by a 92 mm Cambridge pad (Whatman, Maidstone, UK) using a Borgwaldt RM 20 smoking machine (Hamburg, Germany) under smoking conditions as recommended by the US Federal Trade Commission (FTC) [11], or International Standard Organization (ISO) [12–14]. FTC conditions require a puff volume of 35 mL, a puff interval of 60 s, and a puff duration of 2 s. The airflow at the cigarette level in the machine was typically 125±30 mm/s. Using ISO conditions, the airflow was controlled at 200±30 mm/s, and the puff volume, interval and duration remained the same as the FTC values. Unless noted in the text, the smoking conditions were set to the FTC guidelines.

2.3. Sample preparation

Smoking and sample preparation were performed on the same day. After smoking, the Cambridge pad was placed in a screw-cap glass jar and then spiked with 100 μL of the I.S. solution and extracted with 100 mL of 5% HCl using a wrist-action shaker for 30 min. The low pH of the HCl extract ensured that the aromatic amines were positively charged through ionization of the amine group. A filtered 2 mL aliquot of the HCl extract was used for the RapidTrace workstation (Zymark, Hopkinton, MA, USA) fitted with unconditioned 3 mL 60 mg Waters Oasis MCX cartridges. The polymeric MCX cartridges were designed with a cationic-exchange mechanism using benzenesulfonic acid groups. Non-polar rings in the polymer matrix also provided a less-selective retention mechanism. After rinsing the MCX cartridge with 1% HCl and methanol, the compounds retained on the MCX were eluted with 2 mL of 5% NH_4OH in methanol. The first SPE step removed

many neutral and polar compounds from the initial HCl extract.

With the pH change from the HCl extract to the NH_4OH in methanol eluent, the aromatic amines went from charged to neutral, and were thereby ready for the second SPE step using nonpolar/hydrophobic retention. The second SPE cartridge was a 3 mL 60 mg Waters Oasis HLB (hydrophilic–lipophilic balance). Both MCX and HLB SPE cartridges were made from a polymeric material providing stability over the pH range from 0 to 14.

Ten milliliters of water adjusted to pH 11 with NaOH were added to the methanol eluent to modify the sample to a higher water content adequate for the reversed-phase HLB cartridge. The HLB cartridge was conditioned with 3 mL of methanol followed by 2 mL of water (pH 11). The diluted eluent was then applied to the HLB cartridge to retain the aromatic amines. After sample loading, the cartridge was rinsed with 2 mL of water (pH 11) and then rinsed with 2 mL of methanol–water (30:70, v/v) to remove some less-hydrophobic compounds. By increasing the percent of methanol in the rinse, a cleaner eluent would be achieved, but the more-polar aromatic amines can be lost. The 30:70 ratio was a compromise between good retention of the aromatic amines and removing additional hydrophobic compounds. After the rinses, the cartridge was dried using nitrogen. The aromatic amines were eluted from the HLB cartridge using 1.5 mL of toluene. The toluene eluent was transferred to an autosampler vial, and 4 μL of the derivatization reagent, heptafluorobutyric anhydride (HFBA), was added. After incubation at 80 °C for 30 min, the sample was ready for analysis by GC–MS. It was not necessary to consume the excess HFBA by the addition of an alcohol or other reactants. The derivatization procedure was based on that previously reported by Forehand et al. [15] and systematically described in Ref. [16]. Once derivatized, the samples could be stored for at least 1 week at room temperature in a dark container prior to analysis by GC–MS. A flow diagram of the sample preparation steps is presented in Fig. 1.

The results from the automated RapidTrace workstation were confirmed with a manual SPE manifold. A 4 mL aliquot was used for manual SPE extraction on a 12-port vacuum manifold with 6 mL 150 mg MCX or 6 mL 200 mg HLB SPE cartridges.

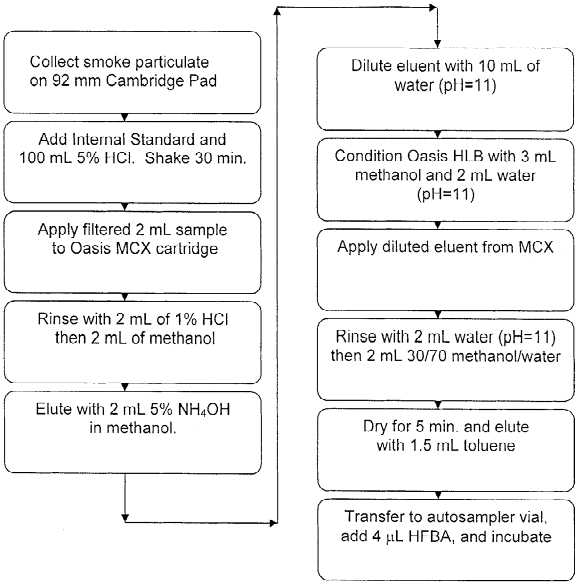

Fig. 1. Schematic flow chart of the SPE procedure.

Additional amines that are present in cigarette smoke, such as 2-methyl-1-naphthylamine, with similar polarity and molecular mass can be quantified by the method presented here.

2.4. Sample analysis

The sample analysis was performed on an Agilent 6890/5973 GC–MS system. The GC system was equipped with a 30 m×0.25 mm I.D. and 0.25 μm film thickness HP-5 capillary column (or an equivalent). The oven was programmed at an initial temperature of 50 °C for 2 min, heated to 205 °C at 8 °C/min, then to 310 °C at 28 °C/min with a final hold time of 3 min. The injector was operated in the split mode (1:10) with a split flow of 1.5 mL/min. The injector temperature was set at 300 °C. The mass spectrometer was operated in the SIM (selected ion monitoring) mode with the ion source configured for negative chemical ionization (NCI) using methane as reagent gas at 2 mL/min flow. For quantitation, the negative chemical ionization of the aromatic amine–HFBA derivatives produced the strongest signals from loss of HF [M−20], as listed in Table 1.

The response factor for each analyte was obtained by injecting known amounts of standards into the

Table 1
Ions from the HFBA derivatives used in the SIM mass spectra acquisition in the NCI mode. The analytes are listed in order of appearance in the chromatogram shown in Fig. 2

No.	Aromatic amine	Ion m/z	Remark
1	[$^2H_5$]Aniline	274	I.S.
2	Aniline	269	Analyte
3	[$^2H_9$]*o*-Toluidine	290	I.S.
4	*o*-Toluidine	283	Analyte
5	*m*-Toluidine	283	Analyte
6	*p*-Toluidine	283	Analyte
7	2-Ethylaniline	297	Analyte
8	2,6-Dimethylaniline	297	Analyte
9	2,5-Dimethylaniline	297	Analyte
10	2,4-Dimethylaniline	297	Analyte
11	3-Ethylaniline	297	Analyte
12	3,5-Dimethylaniline	297	Analyte
13	2,3-Dimethylaniline	297	Analyte
14	4-Ethylaniline	297	Analyte
15	3,4-Dimethylaniline	297	Analyte
16	1-Aminonaphthalene	319	Analyte
17	2-Aminobiphenyl	345	Analyte
18	[$^2H_7$]2-Aminonaphthalene	326	I.S.
19	2-Aminonaphthalene	319	Analyte
20	3-Aminobiphenyl	345	Analyte
21	[$^2H_9$]4-Aminobiphenyl	354	I.S.
22	4-Aminobiphenyl	345	Analyte
23	[$^2H_8$]Benzidine	564	I.S.
24	Benzidine	556	Analyte
25	Tolidine	584	Analyte

GC–SIM-MS system and calculating the ratio of the analyte peak area to its internal standard peak area on an equal mass basis. To calculate the amount of analyte in a sample, the peak area ratio of the analyte and labeled standard was divided by the response factor and then multiplied by the amount of labeled standard added to the sample. This result is then divided by the number of cigarettes smoked to produce the amount of analyte per cigarette.

3. Results and discussion

Using the SPE method, the single ion chromatograms indicated that each analyte was easily identified and no significant interferences were found that might complicate the identification and quantitation from smoking samples. The analytes were well resolved, except for 2,6- and 2,5-dimethylaniline where the peaks were approximately 50% resolved. Additional aromatic amines can be included in the list of target analytes as they are identified and standards purchased for confirmation.

The chromatogram in Fig. 2 was obtained from the extract of 20 1R4F Kentucky reference cigarettes with the internal standards added for quantitation. It can be seen that the SPE method produced excellent cleanup from the endogenous amounts of aromatic amines in the particulate phase of 1R4F cigarette smoke. For Carlton 1 mg cigarettes, which produce very small amounts of particulate phase material, the smaller amounts of aromatic amines/cigarette did not create difficulty in their determination.

3.1. Recovery study

Of the characteristics of an analytical sample preparation methodology using isotopically labeled internal standards, recovery has the most flexible interpretation of what is acceptable. This arises from the fact that the labeled internal standard is effective in accounting for significant variability and analyte losses. Among these are changes in derivatization time and temperature, lot-to-lot variations in the SPE cartridges, poor recovery, etc. It is frequently possible to obtain accurate results with 5% recovery of the analytes. However, a recovery of 60% is commonly viewed as the minimum for a validated method whether labeled internal standards are used or not. When the analyte amounts approach the method detection limit, highly reproducible recoveries become necessary to avoid false-negative results. The results for recovery of this aromatic amine method are presented in Table 2, the compounds being listed as they appear in the order of their retention times in the chromatograms. The recovery ranged from 79 to 109%, well above the needed threshold.

For the recovery evaluation, the amount of each aromatic amine in the particulate phase of Carlton 1 mg cigarette smoke was initially determined. Then 100 ng of each aromatic amine was spiked onto pads containing the particulate phase, and the aromatic amine levels were determined. The change from these two determinations provided the response from the spiked aromatic amines. Finally, 10 μL of a 10 ng/μL aromatic amine standard solution were spiked into the final 1.5 mL extract of the particulate

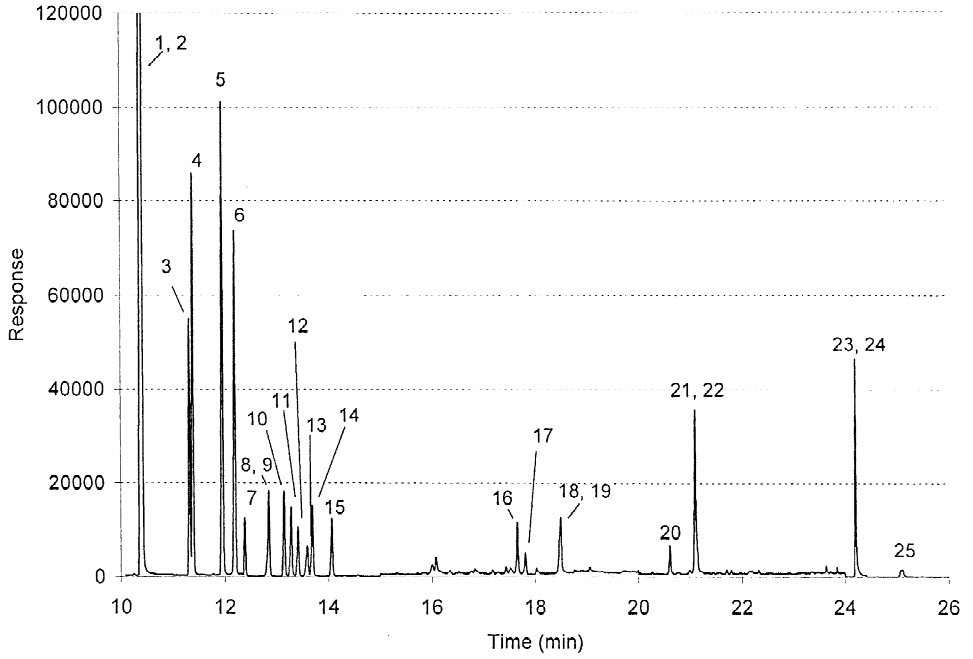

Fig. 2. Reconstructed chromatogram of aromatic amine–HFBA derivatives from the particulate phase of 20 1R4F Kentucky reference cigarettes. The peaks are identified according to the numbering in Table 1.

phase to obtain the response without the sample preparation. The ratio of the aromatic amine response from the sample preparation to the response post-sample preparation was the calculated recovery. Each step was repeated three times to obtain an average. It was found that the GC–SIM-MS analysis of aromatic amines exhibited a better response from the SPE extract than the aromatic amine standards diluted in a plain solvent. The enhanced response was attributed to saturation of ''active sites'' in the GC injector and column by matrix components from the pad extract. The pad extracts had additional components that could react with the active sites and prevent the loss of aromatic amines.

3.2. Limit of detection (LOD) study

Carlton 1 mg cigarettes were used to obtain the LOD for the method. The SPE extract from one Cambridge pad was analyzed seven times to obtain the standard deviation of the peak height for each aromatic amine. The calculated variance was generated exclusively from the errors in the GC–SIM-MS determination. The LOD was then reported as three times the standard deviation (SD) [17]. The results for the LOD are presented in Table 3 with the average aromatic amine amount, SD, and the relative standard deviation (RSD).

The values for the LOD indicate that the SPE–GC–SIM-MS protocol was very sensitive and would be able to determine aromatic amines in a wide variety of cigarette types. The large RSD value for 2,6-dimethylaniline was due to uncertainty in the integration of the peak, as it occurred as a shoulder on the 2,5-dimethylaniline peak. Also, the RSD for tolidine was large since the particulate phase of Carlton 1 mg cigarette smoke contained very low levels that were just above the baseline noise. However, both 2,6-dimethylaniline and tolidine had small absolute standard deviations, indicating that there were no problems with the SPE methodology.

3.3. Precision study

The precision of the SPE steps was examined by analyzing five aliquots from the same 5% HCl pad

Table 2
Percent recovery for the aromatic amines using the SPE method

Aromatic amine	Recovery (%)
[$^2H_5$]Aniline	80
Aniline	79
[$^2H_9$]*o*-Toluidine	102
o-Toluidine	101
m-Toluidine	109
p-Toluidine	106
2-Ethylaniline	103
2,6-Dimethylaniline	80
2,5-Dimethylaniline	109
2,4-Dimethylaniline	101
3-Ethylaniline	99
3,5-Dimethylaniline	101
2,3-Dimethylaniline	99
4-Ethylaniline	98
3,4-Dimethylaniline	94
1-Aminonaphthalene	104
2-Aminobiphenyl	101
[$^2H_7$]2-Aminonaphthalene	89
2-Aminonaphthalene	101
3-Aminobiphenyl	101
[$^2H_9$]4-Aminobiphenyl	99
4-Aminobiphenyl	105
[$^2H_8$]Benzidine	79
Benzidine	80
Tolidine	105

extract of the particulate phase from Carlton 1 mg cigarettes. In this manner, the variance from the MCX and HLB cartridges plus that from the integration could be examined. The results for each replicate, the average, standard deviation and RSD are shown in Table 4. The precision of the SPE steps was very good for most analytes, noting that 2,6-dimethylaniline and tolidine had large RSDs, due to the reasons mentioned previously. If a full-flavor cigarette that had larger amounts of aromatic amines was studied, it would be expected that the variance should decrease for 2,6-dimethylaniline and tolidine.

Through a comparison of Tables 3 and 4, the contribution to the variance from the SPE can be estimated. In Table 3 the same sample was injected seven times into the GC–SIM-MS system and the calculated variance was exclusively from the errors in the GC–SIM-MS determination. In Table 4, the variance was caused by both the sample preparation and the GC–SIM-MS. Overall, the sample preparation contributed one-half of the total variance with a few exceptions. It was interesting to note that the sample preparation steps on the Zymark RapidTrace workstation produced a variance of nearly equal

Table 3
Average amount from a Carlton 1 mg cigarette, standard deviation (SD), limit of detection (LOD) and relative standard deviation (RSD) for each aromatic amine

Aromatic amine	Average (ng/cigarette)	SD (ng/cigarette)	LOD (ng/cigarette)	RSD (%)
Aniline	69.29	0.47	1.41	0.68
o-Toluidine	10.59	0.13	0.40	1.27
m-Toluidine	22.51	0.19	0.56	0.83
p-Toluidine	25.06	0.25	0.75	1.00
2-Ethylaniline	3.94	0.09	0.28	2.35
2,6-Dimethylaniline	0.10	0.02	0.06	20.62
2,5-Dimethylaniline	3.64	0.09	0.27	2.44
2,4-Dimethylaniline	3.29	0.08	0.24	2.40
3-Ethylaniline	5.93	0.09	0.27	1.54
3,5-Dimethylaniline	3.11	0.19	0.58	6.23
2,3-Dimethylaniline	0.39	0.02	0.07	6.38
4-Ethylaniline	8.27	0.34	1.01	4.07
3,4-Dimethylaniline	3.58	0.11	0.34	3.18
1-Aminonaphthalene	4.87	0.05	0.16	1.12
2-Aminobiphenyl	0.45	0.01	0.03	2.49
2-Aminonaphthalene	8.82	0.13	0.38	1.44
3-Aminobiphenyl	0.92	0.05	0.15	5.36
4-Aminobiphenyl	0.60	0.02	0.05	2.64
Benzidine	0.20	0.02	0.05	9.36
Tolidine	0.03	0.01	0.02	19.74

Table 4
Method precision from five replicates from Carlton 1 mg cigarettes of the same 5% HCl Cambridge pad extract

Aromatic amine	Average (ng/cigarette)	SD (ng/cigarette)	RSD (%)
Aniline	67.28	1.46	2.17
o-Toluidine	10.58	0.08	0.79
m-Toluidine	22.14	0.33	1.48
p-Toluidine	25.12	2.42	9.62
2-Ethylaniline	3.95	0.42	10.71
2,6-Dimethylaniline	0.08	0.02	24.22
2,5-Dimethylaniline	3.55	0.26	7.26
2,4-Dimethylaniline	3.14	0.17	5.52
3-Ethylaniline	5.83	0.15	2.55
3,5-Dimethylaniline	2.98	0.09	2.88
2,3-Dimethylaniline	0.35	0.02	5.99
4-Ethylaniline	8.11	0.57	7.07
3,4-Dimethylaniline	3.40	0.17	4.93
1-Aminonaphthalene	4.96	0.13	2.53
2-Aminobiphenyl	0.44	0.03	6.56
2-Aminonaphthalene	8.57	0.26	2.98
3-Aminobiphenyl	0.90	0.11	12.27
4-Aminobiphenyl	0.61	0.05	8.92
Benzidine	0.16	0.03	18.83
Tolidine	0.01	0.01	63.31

value to that produced by the Agilent 6890/5973 GC–MS system (operated in the SIM NCI mode).

3.4. Linearity of calibration

The amounts of aromatic amines per 1R4F cigarette were reported to vary from 1.3 ng for tolidine to as much as 212 ng for aniline, making it necessary to examine the response generated by the aromatic amines over a wide range [15]. We chose to use deuterium-labeled internal standards to examine the linearity from 0.5 to 500 ng/cigarette. This avoided the problem caused by spiking aromatic amines onto Cambridge pads already containing aromatic amines from the particulate phase. The experiment entailed spiking internal standards at different amounts onto Cambridge pads containing the particulate phase of 20 Carlton 1 mg cigarettes and processing the pads through the SPE–GC–SIM-MS protocol. The mass spectrometer was tuned and calibrated, and then the samples and blanks were analyzed consecutively on the GC–MS system so that the GC–MS response would be stable. The peak area was calculated for

Table 5
Results for 1R4F and 2R4F Kentucky reference cigarettes obtained using the SPE method and ISO smoking conditions

Aromatic amine	1R4F		2R4F	
	Average (ng/cigarette)	SD (ng/cigarette)	Average (ng/cigarette)	SD (ng/cigarette)
1-Aminonaphthalene	17.44	0.64	17.00	1.26
2-Aminonaphthalene	9.50	0.24	8.60	0.68
3-Aminobiphenyl	3.38	0.08	2.95	0.30
4-Aminobiphenyl	2.07	0.04	1.60	0.13
2,4-Dimethylaniline	19.06	1.51	15.12	2.16
2,5-Dimethylaniline	15.54	1.17	12.46	1.91
2,6-Dimethylaniline	6.28	0.63	3.93	0.53
2-Ethylaniline	8.75	0.67	6.84	0.89
3,4-Dimethylaniline	13.32	0.59	8.23	1.17
3,5-Dimethylaniline	9.05	0.64	6.77	1.07
3-Ethylaniline	10.09	0.74	7.96	1.25
4-Ethylaniline	8.75	0.59	6.48	1.02
Aniline	331.40	16.88	251.60	18.09
Benzidine	0.11	0.02	0.09	0.02
m-Toluidine	55.22	0.57	46.26	4.71
o-Toluidine	49.76	0.59	42.42	2.72
p-Toluidine	41.56	1.57	29.68	3.23
Tolidine	0.03	0.02	ND	
Total amines	601.30		467.99	

ND, not detectable.

each internal standard and was plotted against the spiked amount. Each of the five internal standard responses exhibited a linear behavior over the entire range, and the correlation was excellent with the lowest r^2 value being 0.997.

3.5. Accuracy

The results for 1R4F and 2R4F Kentucky reference cigarettes obtained using the new SPE method and ISO smoking conditions are given in Table 5. These data were compared to literature values for similar cigarettes and are compiled in Table 6 for the commonly analyzed aromatic amines. There was only one direct comparison with Ref. [15] for all analytes for the 1R4F cigarette, and data on aminonaphthalene and aminobiphenyl were found in Refs. [3,18]. Data for the 1R4F and 2R4F cigarettes from Ref. [18] are the average values reported from four laboratories in a collaborative study performed using ISO conditions. The values from the additional references were not based on the 1R4F cigarette. When compared with the published data for 1R4F and similar cigarettes, the aromatic amine amounts obtained from this work using 1R4F cigarettes fell into the expected range and were in close agreement with Ref. [18].

A further comparison was performed for 1R4F Kentucky reference cigarettes, which were analyzed by two techniques, the one described in this report and by applying in our laboratory the steam distillation extraction method as described in Ref. [15]. We performed both techniques during the same week and with the same I.S. solution. The results from these two techniques are presented in Table 7. As seen in the table, the results of the two methods may differ by more than 10–15% for certain analytes. This can be attributed to the problem of steam distillation extraction wherein the method may not have equal

Table 7
Comparison of results in ng/cigarette for Kentucky reference 1R4F cigarettes that were analyzed by the present method and by applying in our laboratory the method using steam distillation extraction (SDE) as described in Ref. [15]. Both methods were performed during the same week

Aromatic amine	Method		
	SPE	SDE	Difference (%)
1-Aminonaphthalene	17.44	16.76	4
2-Aminonaphthalene	9.50	9.24	3
3-Aminobiphenyl	3.38	4.76	−29
4-Aminobiphenyl	2.07	3.24	−36
2,4-Dimethylaniline	19.06	12.68	50
2,5-Dimethylaniline	15.54	11.04	41
2,6-Dimethylaniline	6.28	4.64	35
2-Ethylaniline	8.75	9.32	−6
3,4-Dimethylaniline	13.32	8.58	55
3,5-Dimethylaniline	9.05	5.26	72
3-Ethylaniline	10.09	6.46	56
4-Ethylaniline	8.75	8.38	4
Aniline	331.40	336.88	−2
Benzidine	0.11	ND	–
m-Toluidine	55.22	43.08	28
o-Toluidine	49.76	70.62	−30
p-Toluidine	41.56	29.22	42
Tolidine	0.03	ND	–
Total amines	601.30	580.16	4

ND, not detectable.

Table 6
Comparison of aromatic amine amounts in ng/cigarette from 1R4F cigarettes using the method reported herein to literature values for similar cigarettes

Aromatic amine	Citation and cigarette type						
	This work (1R4F)	Ref. [15] (1R4F)	Ref. [8] (Camel)	Ref. [6] (USA 85 mm)	Ref. [10] (1R4F)	Ref. [18] (1R4F)	Ref. [18] (2R4F)
Aniline	331.4	212.4	220	102			
o-Toluidine	49.8	39.8	41.5	32.2			
1-Aminonaphthalene	17.4	9.3	5.57	4.3		15.6	15.1
2-Aminonaphthalene	9.5	9.8	3.82	1.0	12.1	10.4	10.3
3-Aminobiphenyl	3.4	6.3	0.47	2.7		3.2	3
4-Aminobiphenyl	2.1	5.4	0.3	2.4	3.3	1.9	1.7
Benzidine	0.11	2.2					
Tolidine	0.03	1.3					

recoveries for the analytes and their deuterated internal standards.

4. Conclusion

An analytical method to determine the levels of aromatic amines in the particulate phase of cigarette smoke has been developed. The method was automated on a Zymark RapidTrace workstation and exhibited very good recoveries, reproducibility, LOD and lack of interferences. The results for 1R4F and 2R4F Kentucky reference cigarettes are reported. The data from this report are in agreement with data from the literature regarding the levels of aromatic amines delivered by the 1R4F cigarette.

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