Determination of Dibenzacridines in the Particulate Phase of Cigarette Smoke*

by

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SUMMARY

This study attempted to resolve a controversy related to the presence of dibenz[*a*,*j*]acridine and dibenz[*a*,*b*]acridine in the particulate phase of cigarette smoke. Smoking was performed using FTC conditions (35 mL puff volume, 2 s puff, 1 min interval) on a Borgwaldt RM 20/CS smoking machine. The particulate phase of forty cigarettes was collected on 92 mm Cambridge filter pads. Pads were combined to analyze the particulate phase of the mainstream smoke from between 120 and 320 cigarettes. In an initial scheme of analysis, the pads were extracted with an acidic aqueous solution. This aqueous solution was then washed with CH₂Cl₂ and the organic phase discarded. The aqueous solution was then changed to basic and extracted with CH₂Cl₂, which was concentrated and analyzed via GC/MS. The dibenzacridine could not be detected utilizing this scheme, even when the pads had been spiked with a few thousand nanograms of dibenzacridine. After using several other organic solvents (cyclohexane, CHCl₃, and benzene) to eliminate the possibility that the extraction efficiency of CH₂Cl₂ was poor, it was determined that dibenzacridine was being discarded with the first CH₂Cl₂ wash. A successful separation scheme was developed by extracting the smoked pad with an aqueous acidic solution, followed by extraction of the aqueous phase with CH₂Cl₂ without pH change. The CH₂Cl₂ extract was concentrated under nitrogen and 1 µL injected for GC/MS analysis. Quantification was achieved by spiking the pads with dibenz[*a*,*j*]acridine-*d*₁₃ as an

internal standard at a level equal to 1.7–2.5 ng/cig. The limit of detection for this technique was approximately 0.5 ng/cig. The chromatographic separation was performed with a 30 m BPX-5 column (0.25 mm i.d., 0.25 µm film thickness). Mass spectral data were acquired in selected ion monitoring (SIM) mode with $m/z = 279$ for the two dibenzacridine isomers and $m/z = 292$ for the deuterated internal standard. Three commercial cigarettes were analyzed: two commercial Full Flavor cigarettes, one commercial Light cigarette, and a 1R4F reference cigarette. Neither dibenzacridine isomer was detected in any of the samples analyzed. [Beitr. Tabakforsch. Int. 19 (2000) 25–31]

ZUSAMMENFASSUNG

Diese Studie soll ein Beitrag zur Lösung einer Kontroverse bezüglich der Anwesenheit von Dibenz[*a*,*j*]acridin und Dibenz[*a*,*b*]acridin in der Partikelphase des Zigarettenrauchs sein. Das Abrauchen der Zigaretten erfolgte nach FTC-Bedingungen (Zugvolumen 35 mL, Zugdauer 2 Sek., Zugintervall 1 Min.) mit einer Borgwaldt RM 20/CS Rauchmaschine. Die Partikelphase von je 40 Zigaretten wurde auf 92 mm Cambridgefiltern gesammelt. Zur Analyse der Partikelphase des Hauptstromrauchs von 120 bis 320 Zigaretten wurden mehrere Cambridgefilter untersucht. In einem ersten Schritt wurden die Cambridgefilter mit einer sauren wässrigen Lösung extrahiert. Diese wässrige Lösung wurde anschließend mit CH₂Cl₂ gewaschen, und die organische

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Phase wurde verworfen. Die wässrige Lösung wurde dann alkalisch gemacht und mit CH_2Cl_2 extrahiert, das konzentriert und mittels GC/MS analysiert wurde. Bei diesem Verfahren konnte kein Dibenzacridin nachgewiesen werden, auch dann nicht, wenn den Cambridgefiltern mehrere Tausend Nanogramm Dibenzacridin zugesetzt wurde. Nach Verwendung mehrerer anderer organischer Lösungsmittel (Cyclohexan, CHCl_3 und Benzol), um die Möglichkeit auszuschließen, dass die Extraktionsfähigkeit von CH_2Cl_2 zu gering ist, wurde gefunden, dass das Dibenzacridin beim ersten CH_2Cl_2 -Waschvorgang verloren ging. Es wurde ein erfolgreiches Trennungungsverfahren entwickelt, bei dem der Cambridgefilter mit einer sauren wässrigen Lösung extrahiert wurde, gefolgt von der Extraktion der wässrigen Phase mit CH_2Cl_2 ohne pH-Änderung. Anschließend erfolgte Konzentration unter Stickstoff und Injektion von 1 μL der Lösung für die GC/MS-Analyse. Für die quantitative Bestimmung wurde den Cambridgefiltern als interner Standard Dibenz[*a,j*]acridin- d_{13} in einer Menge zugesetzt, die 1,7–2,5 ng/Cig. entspricht. Die Nachweisgrenze für dieses Verfahren betrug etwa 0,5 ng/Cig. Die chromatographische Auftrennung erfolgte mittels einer 30 m BPX-5-Säule (Innendurchmesser 0,25 mm, Filmdicke 0,25 μm). Die Massenspektraldaten wurden im SIM-Betrieb (selected ion monitoring) unter Verwendung von $m/z = 279$ für die beiden Dibenzacridin-Isomere und $m/z = 292$ für den deuterierten internen Standard gemessen. Es wurden drei handelsübliche Cigaretten analysiert: zwei handelsübliche Full Flavour Cigaretten, eine handelsübliche Leichtcigarette und eine 1R4F-Vergleichscigarette. In keiner der analysierten Proben wurde eines der Dibenzacridin-Isomere nachgewiesen. [Beitr. Tabakforsch. Int. 19 (2000) 25–31]

RESUME

Le but de cette étude est d'élucider la controverse relative à la présence de dibenzo[*a,j*]acridine et de dibenzo[*a,b*]acridine dans la phase particulaire de la fumée de cigarette. Les cigarettes ont été fumées sur une machine à fumer Borgwaldt RM 20/CS selon les conditions de la Federal Trade Commission (FTC) (volume de bouffée: 35 mL, durée de bouffée: 2 secondes, fréquence de bouffée: 1 minute). La phase particulaire de 40 cigarettes a été recueillie sur des filtres Cambridge de 92 mm. Un ensemble de plusieurs filtres Cambridge, sur lesquels était recueillie la phase particulaire de la fumée du courant principal de 120 à 320 cigarettes, a été analysé. Dans un premier temps, l'extraction des filtres avait été réalisée avec une solution aqueuse acide, qui était ensuite lavée au CH_2Cl_2 , la phase organique étant alors jetée. La solution aqueuse était ensuite changée en solution basique, et l'extraction se faisait au CH_2Cl_2 , avec concentration et analyse par GC/MS. La dibenzoacridine n'était pas détectée par cette méthode, de même

lorsque les filtres Cambridge étaient imprégnés de quelques milliers de nanogrammes de dibenzoacridine. A la suite de l'utilisation de plusieurs autres solvants organiques (cyclohexane, CHCl_3 , et benzène) pour éliminer la possibilité de l'inefficacité de l'extraction au CH_2Cl_2 , il a été déterminé que la dibenzoacridine était rejetée par le premier lavage au CH_2Cl_2 . Une méthode satisfaisante de séparation a été mise au point, consistant en une extraction des filtres Cambridge avec une solution acide aqueuse, suivi par l'extraction de la phase aqueuse au CH_2Cl_2 sans changer le pH, puis par une concentration à l'azote et l'injection de 1 μL de la solution pour l'analyse par GC/MS. La quantification a été réalisée en imprégnant les filtres Cambridge avec de la dibenzo[*a,j*]acridine- d_{13} comme étalon interne, et à une quantité qui correspondait à 1,7–2,5 ng/cig. La limite de détection de cette technique était d'environ 0,5 ng/cig. La séparation chromatographique a été réalisée à l'aide d'une colonne BPX-5 de 30 m (diamètre intérieur = 0,25 mm, épaisseur du film = 0,25 μm). Les données d'analyse par spectrométrie de masse ont été obtenues en mode de contrôle ionique spécifique (SIM = selected ion monitoring), avec $m/z = 279$ pour les deux isomères de dibenzoacridine et $m/z = 292$ pour le standard deutéré interne. Trois cigarettes vendues dans le commerce ont été analysées: deux cigarettes plein arôme, une cigarette légère et une cigarette de référence Kentucky 1R4F. Aucun des deux isomères de dibenzoacridine n'a été détecté dans les échantillons analysés. [Beitr. Tabakforsch. Int. 19 (2000) 25–31]

INTRODUCTION

Dibenz[*a,j*]acridine and dibenz[*a,b*]acridine have been reported as present in smoke condensate in a 1960 publication by VAN DUUREN *et al.* (1). Also, unpublished data of CANDELI *et al.* were reported by WYNDER and HOFFMANN (2) in 1967, who again indicated the presence of these compounds in smoke. In a list of tobacco smoke components published in 1998 by HOFFMANN and HOFFMANN (3), dibenz[*a,j*]acridine was included as being present at 3–10 ng/cig. However, as reported by RODGMAN (4) in 1998, a series of seven independent studies performed by KABURAKI *et al.* (5), SCHMELTZ *et al.* (6, 7), SNOOK *et al.* (8, 9), GRIMMER *et al.* (10), and KAMATA *et al.* (11), failed to find this substance in smoke. The controversy about the presence of dibenzacridines in smoke is also discussed in a recent monograph on tobacco (12). The work presented here is intended to resolve the problem and attempted to detect the presence of dibenzacridines in the particulate phase of cigarette smoke. In their 1960 study, VAN DUUREN *et al.* (1) also reported the presence of dibenzocarbazole in cigarette smoke. After some preliminary investigation, the present study shows that it is not possible to detect this substance with Van Duuren's procedure.

EXPERIMENTAL METHOD

Materials

All solvents utilized were spectral grade. Dibenz[*a,j*]acridine, dibenz[*a,b*]acridine, and 7*H*-dibenzo[*c,g*]carbazole were purchased from Promochem (Laramie, W.Y., USA). Dibenz[*a,j*]acridine-*d*₁₃ and 7*H*-dibenzo[*c,g*]carbazole-*d*₁₂ were used as internal standards and both were purchased from Cambridge Isotope Laboratories, Inc. (Andover, M.A., USA).

Cigarette samples

The mainstream smoke from three commercial cigarettes and 1R4F reference cigarettes were analyzed. All cigarettes were king size and filtered. Commercial cigarette A was a Full Flavor cigarette and commercial cigarette B was a Light. They reportedly yield 17.5 mg and 11.1 mg total particulate matter (TPM), respectively. Commercial cigarette C was a Full Flavor, 100% natural tobacco product with a reported TPM value of 17.4 mg. The reported TPM value for 1R4F reference cigarettes is 11.0 mg.

Smoking and TPM collection

All smoking was done under FTC specifications (35 mL puff volume, 2 s puff duration, 1 min interval)

on a Borgwaldt RM 20/CS smoking machine. The TPM from forty cigarettes was collected on a single 92 mm Cambridge filter pad. Pads were combined to yield TPM from a total of between 120 and 320 cigarettes.

Sample extraction and preparation

The initial extraction procedure started with smoking 40 cigarettes onto a Cambridge pad. The pad was spiked with 4000 ng of a standard solution of dibenz[*a,j*]acridine to determine if, and to what efficiency, the analyte would be extracted with this procedure. After spiking, the pad was extracted three times with 50 mL of a 0.1 N HCl solution in water. The extraction was done on a wrist shaker for 20 minutes and the liquid was vacuum filtered. Then, the acidic aqueous solution was washed two times with about 20 mL CH₂Cl₂ and the organic phase was discarded. After this clean-up step, the pH of the solution was changed to basic with NaOH, followed by extraction of this aqueous solution three times with 10 mL CH₂Cl₂. The CH₂Cl₂ extracts were combined and evaporated under a stream of nitrogen to a final volume of 1 mL and 1 μL of this solution was injected into the GC/MS for analysis. This scheme (Scheme A) is shown in Figure 1. Scheme A was unsuccessful in extracting the standard from a smoke pad. It was determined that the analyte was getting discarded with the first CH₂Cl₂ wash, so

Figure 1.
Initial scheme for extraction of dibenzacridines from Cambridge pads.

Figure 2.
Revised scheme for extraction of dibenzacridines from Cambridge pads.

the extraction procedure was modified. The modified technique, Scheme B, is shown in Figure 2. The main differences between Schemes A and B are the omission of the first CH_2Cl_2 wash as well as the step changing the pH of the aqueous solution from acidic to basic.

Instrumentation

Chromatography. All separations were performed on a 30 m BPX-5 column (SGE, Inc., Austin, TX, USA) with 0.25 mm i.d. and 0.25 μm film thickness housed in an Hewlett Packard (HP) 6890 gas chromatograph. The initial temperature was 45 $^\circ\text{C}$, held for 5 minutes, then increased 10 $^\circ\text{C}/\text{min}$ to a final temperature of 310 $^\circ\text{C}$, where it was held for 5 minutes. The instrument was operated in constant flow mode (2.0 mL/min) with helium as the carrier gas.

Detection. An HP 5973 mass selective detector (MSD) was used for detection. It was operated in selected ion monitoring (SIM) mode, monitoring $m/z = 279$ for dibenzacridine and $m/z = 292$ for dibenzacridine- d_{13} .

RESULTS

Extraction method

As previously mentioned, the initial extraction scheme was not successful in extracting the dibenzacridine standards, as they were being discarded with the first organic solvent wash. After the scheme was modified to

Scheme B, the standard was successfully extracted with very good efficiency.

The procedure in Scheme B was applied to various cigarette samples to determine if the standards could be extracted when in a cigarette smoke matrix. Figures 3a and 3b show the extracted ion chromatograms for $m/z = 279$ and $m/z = 292$, respectively, for a sample of 80 cigarettes (commercial cigarette A, 17.5 mg tar) spiked with the equivalent of 10 ng/cig. of each dibenz[a,j]acridine, dibenz[a,b]acridine, and dibenz[a,j]acridine- d_{13} . These figures demonstrate that the extraction procedure is sufficient to analyze dibenzacridines that may be present in the particulate phase of tobacco smoke.

The procedure in Scheme B was also used in our initial attempts to analyze the particulate phase of cigarette smoke for 7H-dibenzo[c,g]carbazole. Scheme B was inadequate for the extraction of dibenzo[c,g]carbazole from the Cambridge pad.

Detection of dibenzacridines in particulate phase tobacco smoke

Several different brands and styles of cigarette smoke were analyzed for the presence of dibenzacridines. To enhance the odds of detecting possible traces of dibenzacridines, up to 320 cigarettes were smoked, the particulate phase collected on eight pads. The pads were combined and extracted by Scheme B. One pad was spiked with the equivalent of 1.7 ng/cig. dibenz[a,j]acridine- d_{13} . Figures 4a and 4b show the respective resulting extracted ion chromatograms for

Figure 3. Extracted ion chromatograms showing separation of the two dibenzacridine isomers and the deuterated compound. The particulate matter from 80 cigarettes were spiked with dibenz[*a,j*]acridine, dibenz[*a,h*]acridine, and dibenz[*a,j*]acridine-*d*₁₃. a) *m/z* = 279; b) *m/z* = 292

$m/z = 279$ and $m/z = 292$. Similar experiments were performed twice for cigarette A and also for commercial cigarettes B and C and a 1R4F cigarette.

Figures 5a and 5b show the mass spectra for dibenz[*a,j*]acridine and dibenz[*a,j*]acridine-*d*₁₃, respectively. The lack of fragmentation in the mass spectra of dibenzacridines is a disadvantage regarding the presence of confirming ions for identification. However, it is advantageous from a sensitivity standpoint when SIM detection is utilized. The GC/MS technique developed for detection of dibenzacridines in this study was sensitive enough to quantitate as low as 1.7 ng/cig. of dibenz[*a,j*]acridine. It is very likely that detection without quantification can be accomplished at even lower levels.

DISCUSSION

Examination of the various figures presented indicates that the dibenzacridines are not present in smoke at levels higher than 1.5 ng/cig. Also, it is very likely that if present at all in smoke, the level of these compounds is below 0.5

Figure 4. Extracted ion chromatograms of smoke from 320 cigarettes spiked with the equivalent of 1.7 ng/cig. of dibenz[*a,j*]acridine-*d*₁₃. a) *m/z* = 279; b) *m/z* = 292

ng/cig. This finding is in disagreement with the data reported by HOFFMANN and HOFFMANN (3).

Because of this discrepancy with previous results, a more detailed evaluation was made of the initial 1960 paper by VAN DUUREN *et al.*, which indicated the presence of dibenzacridines in cigarette tar. In this paper, the authors started with 250 g of cigarette tar, which is relatively difficult to generate. Furthermore, the smoking conditions for generating the tar are not indicated in the original paper. However, the proposed separation by VAN DUUREN *et al.* basically follows Scheme A of the work presented here, which was proven to be inadequate for the analysis of dibenzacridines. In fact, in Scheme A the dibenzacridines were discarded with the organic solvent used to wash the acidic aqueous solution. Therefore, it is highly unlikely that the "basic fraction" separated by the procedure utilized by VAN DUUREN *et al.* can contain dibenzacridines.

Another possible source of ambiguity is the detection/identification procedure utilized by VAN DUUREN *et al.* Identification was based on UV absorption spectra and fluorescence excitation spectra. Although the spectra presented in the paper are indeed similar to those of dibenzacridines, it is generally accepted that these types of spectra are not always conclusive for determining the presence of a specific compound.

Figure 5.
El mass spectra of a) dibenz[*a,j*]acridine and b) dibenz[*a,j*]acridine-*d*₁₃.

7*H*-Dibenzo[*c,g*]carbazole is also reported by VAN DUUREN *et al.* (1) as being detected in the basic fraction of the particulate phase of smoke. No evaluation of possible levels of 7*H*-dibenzo[*c,g*]carbazole was done in this study because neither Scheme A nor Scheme B can be applied for its analysis, as the substance is not a basic compound (13). The verification of extraction path for

dibenzo[*c,g*]carbazole was done by spiking 4000 ng of a dibenzo[*c,g*]carbazole standard on a clean Cambridge pad. After spiking, Scheme A was applied and each step monitored with fluorescence under UV light to check the path of the analyte. As expected, the acidic water solution was unable to extract dibenzo[*c,g*]carbazole from the Cambridge pad. Therefore, a completely

separate extraction scheme is necessary to determine if this compound is present in the particulate phase of smoke and, if so, at what level.

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