Formation of Scopolamine from *N*-Butyl-Scopolammonium Bromide in Cigarettes*

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Abstract

Scopolamine (hyoscine) is a naturally occurring alkaloid found in solonacea, the so-called "night shade" plants. Therapeutic applications of scopolamine are in ophthalmology to cause mydriasis and for the prevention of motion sickness, among others. It is known to induce hallucinogenic effects at a high dose. The *N*-butyl bromide derivative of scopolamine, available commercially as Buscopan®, is commonly used as an antispasmotic. The possibility of forming scopolamine from *N*-butyl-scopolammonium bromide when burning cigarettes fortified with Buscopan was investigated based on a record of a prison inmate who claimed to experience hallucinations after smoking Buscopan. Liquid chromatography–tandem mass spectrometry in electrospray ionization mode was used to monitor the formation of scopolamine. Various series of eight cigarettes spiked with 10 mg of *N*-butyl-scopolammonium bromide with and without filters and in different smoking modes were investigated. The smoke of the burning cigarettes, the ashes, and the filter were analyzed for the presence of scopolamine. Scopolamine was detected in all cases.

Introduction

In January 2006, our laboratory was contacted by the medical staff of a local prison center about a male inmate claiming to have smoked Buscopan® in cigarettes. According to the medical staff, the inmate showed extremely aggressive behavior and hallucinations. Unfortunately, no blood or urine samples were taken at the time and the amount of Buscopan added to the cigarettes was not known. A recent literature search about smoking of Buscopan in cigarettes was unsuccessful and only sparse documentation was found on the Internet. On the *"Erowid"* website, a 21-year-old male named "Woody" described his experience after smoking two cigarettes made from catnip mixed with two crushed Buscopan tablets (1). In particular, his hallucinations comprehended mumbling cavemen, oscillating guitars, purple haze, spiders, and other insects. After the trip, he could not read small print for 1–2 days.

Buscopan, which contains *N*-butyl scopolammonium bromide (BSB, Figure 1), is indicated for the relief of abdominal discomfort, pain, and acute colic (2). As a quaternary ammonium compound with low lipid solubility, BSB cannot pass the blood-brain barrier easily and only rarely causes the central nervous system side effects associated with atropine and scopolamine.

Thus, the question arises whether the hallucinations reported by the prison inmate and "Woody" could not be due to an intoxication with scopolamine, derived from the pyrolysis of BSB in cigarettes. Indeed, scopolamine (Figure 1) overdose is associated with symptoms typical of anticholinergic poisoning, such as gastrointestinal discomfort, tachycardia, facial flushing, dilated pupils, dry skin (3), altered mental status, hallucinations (3,4), and acute delirium (5). Anterograde amnesia and submissive behavior have also been observed in patients intoxicated with scopolamine (6).

The aim of the study was to investigate the possibility of the formation of scopolamine when BSB, the active component of Buscopan, is burned in smoking cigarettes. For this purpose, a smoking machine was designed, and self-made cigarettes, with and without filters, were fortified with crushed Buscopan tablets. Different smoking modes, continuous or puff-by-puff, were simulated. Qualitative and quantitative analyses of the filter, ashes, and smoke were carried out using a newly devel-

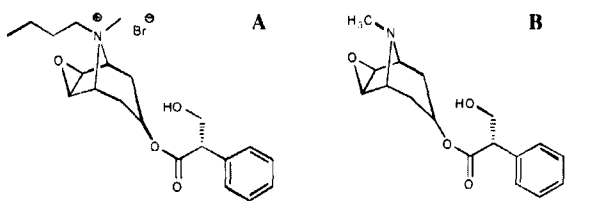

Figure 1. BSB (A) and scopolamine (B).

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oped and validated liquid chromatography–tandem mass spectrometry (LC–MS–MS) method.

Experimental

Chemicals and reagents

Methanol, acetonitrile, and high-performance liquid chromatography (HPLC) grade water were obtained from Lab-Scan Analytical Sciences (Labscan, Ltd., Dublin, Ireland). Buscopan (Boehringer Ingelheim, 50 tablets, each containing 10 mg of BSB) was purchased at a local pharmacy. Scopolamine bromide, BSB, and atropine were kindly provided by the Division du Contrôle des Médicaments of the National Health Laboratory (Luxembourg). Ammonium formate was purchased from Sigma-Aldrich (Steinheim, Germany). Formic acid was purchased from UCB (Brussels, Belgium). Hand-rolling tobacco (Camel®, JT International, Meise, Belgium), empty cigarette tubes with and without cellulose acetate filters (Memphis®, king-size), and a filter tube machine (Bombay®) were purchased at a local market.

Ammonium formate buffer (20mM, pH 3.8) was prepared by dissolving 2.52 g of ammonium formate in 2000 mL of HPLC-grade water and adjusted to pH 3.8 by adding diluted formic acid.

Sample preparation

Ten Buscopan tablets containing 10 mg of BSB each were crushed, and the equivalent quantity of 1 tablet was homogenously mixed with 700 mg of tobacco (the medium amount of tobacco found in cigarettes). The mix was introduced into an empty cigarette tube using the filter tube machine. The length of each cigarette was 58 mm, and the diameter was 8 mm. The length of the filter was 25 mm.

To assess the pyrolysis products, cigarette smoke extracts were collected. The cigarette was connected to a 1000-mL round bottom flask containing 50 mL of a 85:15 mixture of ammonium formate buffer (pH 3.8) and acetonitrile. The round bottom flask was connected to a 1000-mL gas trap filled with 100 mL buffer solution. The whole assembly was connected to a vacuum pump producing a 875 mBar depression. The cigarette was lit, and the cigarette smoke was drawn into the 1000-mL flask where it largely remained. Excessive smoke was collected in the gas trap. After the cigarette was smoked, the flask and the trap were sealed and shaken vigorously until all of the smoke was absorbed by the liquid. The flask and the trap solutions were gathered. The ashes and the filters of the cigarettes were collected and extracted with 8 mL of methanol. After centrifugation for 5 min at 5000 rpm, 40 µL of a 100 mg/L solution of atropine [internal standard (IS)] was added to 4 mL of each of the three matrices (extracted smoke, ashes, and filter). Twenty microliters of each solution was injected into the LC–MS–MS system for analysis.

Three different sets of specimens were prepared using (*i*) cigarettes with filters smoked in 30 s; (*ii*) cigarettes without filters smoked in 30 s; and (*iii*) cigarettes with filters smoked in 2.5 min with intermediary 2 s long puffs every 30 s, to best simulate real smoking conditions. In order to obtain statistically more significant results, each set was repeated eight times.

Instrumental analysis

The analysis of scopolamine was performed on an HPLC system (LC pump P4000 and autosampler AS3000 from Thermo Separation Products) coupled to an LCQ Duo Ion Trap detector (Thermo Electron, Zellik, Belgium) equipped with an ESI interface run in the positive ion mode. The separation of sample components was achieved on an X-Terra MS C18 (3.9 × 150 mm, 5-µm particle size) (Waters, Overijse, Belgium), equipped with an X-Terra MS C18 pre-column (5-µm particle size, 3.9 × 10 mm) and operated at 37°C. Injection volume was 20 µL. The mobile phase consisted of a mixture of 20mM ammonium formate buffer at pH 3.8 (A) and acetonitrile (B). Separation conditions were as follows: 0.0–1.0 min, flow = 0.5 mL/min, A/B hold at 80:20, v/v; 1.0–6.0 min, eluant B increase to 95%; 6.0–7.5 min, eluant B hold at 95%, flow hold at 0.5 mL/min; 7.5–8.5 min, eluant B hold at 95%, flow increase to 1.25 mL/min; 8.5–13.0 min, flow hold at 1.25 mL/min, B = 95%; 13.0–13.5 min, flow = 1.25 mL/min, B to 20%; and 13.5–14.0 min, flow to 0.5 mL/min, B = 20%. Before each run the column was equilibrated for 8 min at a constant flow rate (0.5 mL/min, A/B, 80:20, v/v).

Ionization of the analytes was carried out as follows: sheath gas flow rate (nitrogen), 75 arbitrary units; auxiliary gas flow rate (helium), 10 arbitrary units; spray voltage, 5.0 kV; capillary temperature, 250°C; capillary voltage, 10 V; first octapole offset, –0.75 V; second octapole offset, –6.5 V; and lens voltage, –16.0 V. Data acquisition was performed in a time segment between 2.5 and 7.5 min after injection. The following ions of the full MS–MS spectrum were monitored for scopolamine: parent mass *m/z* 304, isolation width *m/z* 1.6, normalized collision energy 30.0%, mass range *m/z* 125–310 and atropine (IS) *m/z* 290, isolation width *m/z* 1.6, normalized collision energy 32.5%, and mass range *m/z* 120–300.

Validation

Validation experiments included calibration curves, intraday and interday accuracy and precision, and determination of the limit of detection (LOD) and the lower limit of quantification (LLOQ).

Calibration curves

Five-point standard calibration curves were prepared by fortifying a solution of ammonium formate buffer and acetonitrile (85:15, v/v) with known quantities of scopolamine. The final concentrations of scopolamine were 0.5, 1.0, 2.0, 3.0, and 4.0 mg/L. Atropine at a final concentration of 1.0 mg/L was used as the IS. The calibration curve was obtained by plotting the response ratio of scopolamine versus atropine as a function of the concentration of scopolamine. Each calibration point was measured in triplicate.

Precision and accuracy

Intraday accuracy and precision were performed by analyzing the 1.0 and the 4.0 mg/L calibration points of scopolamine four times during the same day. Interday accuracy and precision were evaluated by establishing the calibration curve for scopolamine on four successive days. For acceptance, the relative

standard deviation (RSD) of the precision had to be < 15% and the accuracy had to be in the range of 85% to 115% (7).

LOD and LLOQ

The LOD and the LLOQ were defined as three and six times the level of noise at the same retention time of blank samples, respectively. LOD and LLOQ values were determined using the lowest calibration point for scopolamine.

Results and Discussion

Method development

Standard solutions containing scopolamine, BSB, and atropine were used for analytical method development and validation. A good chromatographic separation of all compounds was achieved (Figure 2).

Before analysis, the mass detection parameters were optimized. For this, a standard solution of scopolamine in a mixture of ammonium formate buffer and acetonitrile (85:15) was injected directly in the MS (without chromatographic separation). The optimization of parameters (capillary voltage, tube lens offset, the first octapole offset, the second octapole offset, and inter-octapole lens) was performed by selecting the value of the apparent molecular ion $[M+H]^+$ for scopolamine and running the automatic optimization program.

Analyte fragmentation was conducted in the MS–MS full scan mode. The full MS–MS spectrum (Figure 3) shows the parent ion of scopolamine ($[M+H]^+$) with a mass-to-charge ratio value of 304. The daughter ions with mass-to-charge ratios of 156 and 138 were generated with a relative collision energy of 30% by loss of the tropic carboxyl group (*m/z* 150) or tropic acid (*m/z* 165). For quantification, the ion with a mass-to-charge ratio of 138 was monitored.

Method validation

Specificity. Blank cigarette extracts were verified to be drug-free. No interferences were found with the studied compounds (scopolamine, BSB, and atropine). Crushed Buscopan tablets, dissolved in the gradient mixture (A/B, 85:15, v/v), were analyzed and contained neither scopolamine nor atropine.

Linearity. The calibration curve for scopolamine was linear over the concentration range of 0.50–4.00 mg/L. The calibration curve was generated using linear regression analysis over the respective concentration range.

The regression equation for scopolamine was $y = 1.914\,x + 0.040$, where y is the concentration expressed in milligrams per liter and x is the peak area ratio, and it showed good linearity (r^2 = 0.993).

Sensitivity. The LOD evaluated for scopolamine was 4 µg/L and the LLOQ was evaluated at 12 µg/L.

Precision and accuracy. Table I shows a summary of intra- and interday precision and accuracy. The intraday accuracy of scopolamine was in the range of 101% to 106% with the precision (RSD) less than 2.7%. The interday accuracy of scopolamine was in the range of 91% to 99% with the precision (RSD) less than 6.9%. These results indicate that the present method has satisfactory accuracy and precision.

Analysis of scopolamine in cigarette extracts

The concentrations obtained from the different matrices were obtained in micrograms per liter in the solvent or in the extraction medium. They were converted to micrograms of scopolamine/cigarette in smoke, filter, and ashes, respectively. The results are summarized in Table II.

In all smoking modes scopolamine was found in the three

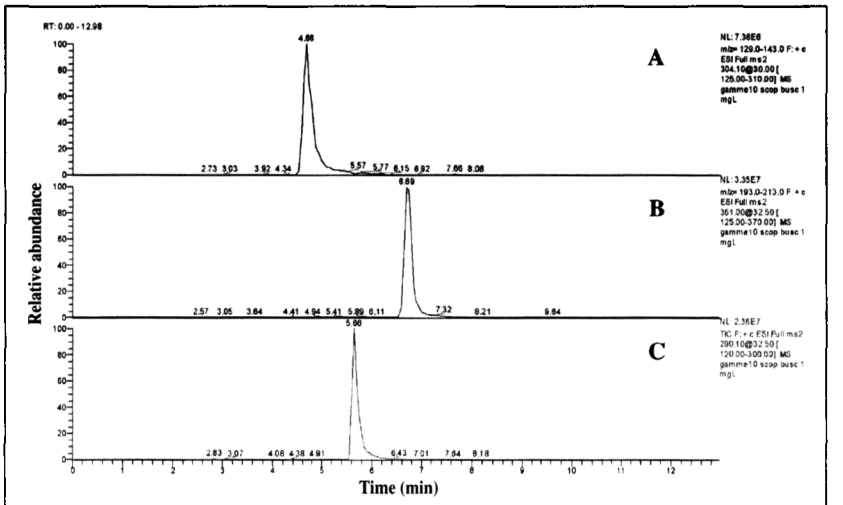

Figure 2. Total ion current chromatograms of scopolamine (retention time = 4.7 min) (A), BSB (retention time = 6.7 min) (B), and atropine (IS; retention time = 5.7 min) (C) at a concentration of 1.0 mg/L.

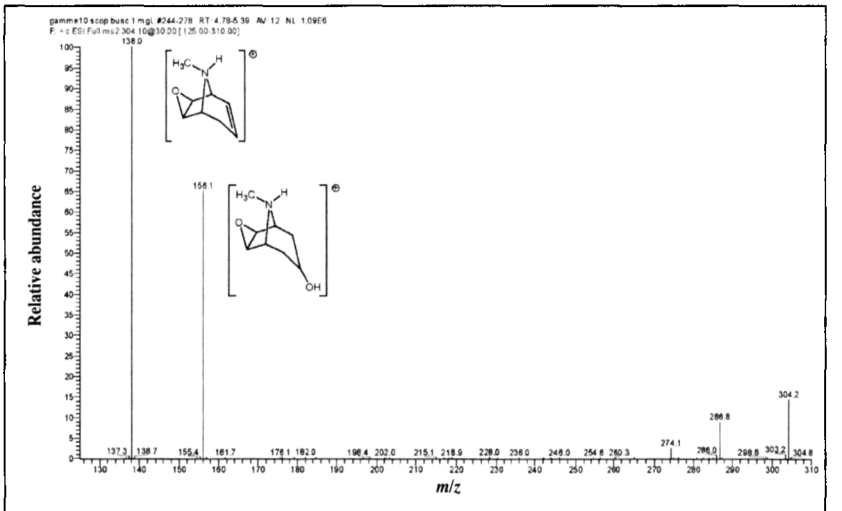

Figure 3. Product ion mass spectrum of scopolamine (MS–MS full scan mode, relative collision energy = 30%) obtained by injection of a standard solution at 1.0 mg/L.

matrices (smoke, filter, and ashes). Quantities of scopolamine in smoke were highest in cigarettes without a filter (range 131–238 µg/cigarette) and lowest in cigarettes with filters, but smoked in the puff-by-puff mode (range 98–166 µg/cigarette). The overall yields of scopolamine formation were 418 µg in cigarettes with filters smoked continuously, 351 µg in cigarettes without filters smoked continuously, and 250 µg in cigarettes with filters smoked puff-by-puff. Thus, a consumer smoking (puff-by-puff) a cigarette spiked with one 10 mg Buscopan tablet potentially inhales scopolamine in the range of 100 to 150 µg. To the best of our knowledge, no data is available that would indicate that this amount of inhaled scopolamine causes central nervous system effects, such as hallucinations as reported by the prison inmate or by "Woody".

The high RSD values in the experiments could be explained by the non-homogeneous distribution of tobacco and the powdered Buscopan tablet in the self-made cigarettes. The resulting variations in the temperature, burning rate, and oxygen concentration influence the chemical processes (pyrolysis, thermal decomposition, and combustion) that take place inside a burning cigarette (8,9). Air currents next to the burning cigarette and the presence or absence of ashes on cone also influence the burning rate, temperature, and oxygen concentration. The burning temperature has been extensively studied and depends on parameters such as tobacco brands (10) and additives (11), but also on the measurement method used (12). The burning zone can be effectively divided into two regions. In the combustion zone, where temperatures reach between 700°C and 900°C, the simple combustion products carbon monoxide, carbon dioxide, water, and heat are released. In the zone just behind the combustion zone, the so-called pyrolysis and distillation zone, temperatures vary between 100°C and 600°C. In this zone, complete combustion is not possible because of a lack of oxygen, but the high temperatures allow pyrolysis and thermal decomposition of the cigarette components. It seems that BSB is decomposed into scopolamine in this zone. Indeed, when BSB was heated to 200–250°C, formation of scopolamine was observed and complete degradation of scopolamine occurred above 300°C (data not shown).

Table I. Precision and Accuracy for LC–MS–MS Analysis of Cigarette Extracts

	Scopolamine		
Target Concentration (mg/L)	Mean (± SD) concentration found (mg/L)	RSD (%)	Accuracy (%)
Intraday (n = 4)			
1.00 mg/L	1.01 ± 0.03	2.7%	101%
4.00 mg/L	4.28 ± 0.11	2.6%	106%
Interday (n = 4)			
1.00 mg/L	0.91 ± 0.06	6.9%	91%
4.00 mg/L	3.95 ± 0.12	3.2%	99%

Table II. Scopolamine Recovered in Smoke, Ashes, and Filter After Smoking Buscopan Spiked Cigarettes Using a Self-Made Smoking Machine

	Continuous Smoking				Puff-by-Puff Smoking	
	With filter mean (n = 8)	RSD	Without filter mean (n = 8)	RSD	With filter mean (n = 8)	RSD
Smoke	169 µg	22%	209 µg	46%	127 µg	20%
Filter	128 µg	30%	–	–	95 µg	15%
Ashes	121 µg	43%	142 µg	15%	28 µg	63%
Total/cigarette	418 µg		351 µg		250 µg	

Conclusions

This study has shown that smoking Buscopan tablets in cigarettes generates scopolamine, a well-known drug causing anticholinergic syndrome (hallucinations, mydriasis, and amnesia). Thus, the rare reports of persons claiming to smoke Buscopan should be taken seriously. Further studies are necessary to elucidate the chemical mechanism of scopolamine formation and to assess the quantity of scopolamine that needs to be absorbed by inhalation to experience CNS effects, such as hallucinations.

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