*Journal of Chromatography*, 528 (1990) 325–331
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5253

# Electron-impact ionization detection of scopolamine by gas chromatography–mass spectrometry in rat plasma and brain

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(First received October 17th, 1989; revised manuscript received February 6th, 1990)

## SUMMARY

Scopolamine, a muscarinic receptor antagonist, was measured in rat plasma and brain using gas chromatography–mass spectrometry with electron-impact ionization detection. Extracted scopolamine was either directly derivatized or first hydrolyzed to scopine, then derivatized with heptafluorobutyric anhydride, separated on a capillary column and quantified by mass fragmentography. Electron-impact ionization produced a common fragment peak at *m/z* 138 that was monitored along with trideuterated scopolamine, an internal standard (*m/z* 141). The method can be used to measure scopolamine concentrations of 2 ng/ml in rat plasma and 20 ng/g in rat brain.

## INTRODUCTION

Scopolamine (Fig. 1a), a natural alkaloid, has a broad spectrum of biological activities. As an antagonist of the muscarinic cholinergic receptor, it has been found to impair memory and, in some cases, to mimic the memory impairment of normal aging [1,2]. In a recent study, scopolamine was administered in low doses to humans with Alzheimer's disease and to age matched control subjects [3]. A greater disruption of cognition occurred in subjects with Alzheimer's disease, suggesting that challenge with scopolamine might be used in diagnosing dementia. Individual responses were quite variable, however, and an attempt to relate response to drug plasma concentrations was not made, possibly

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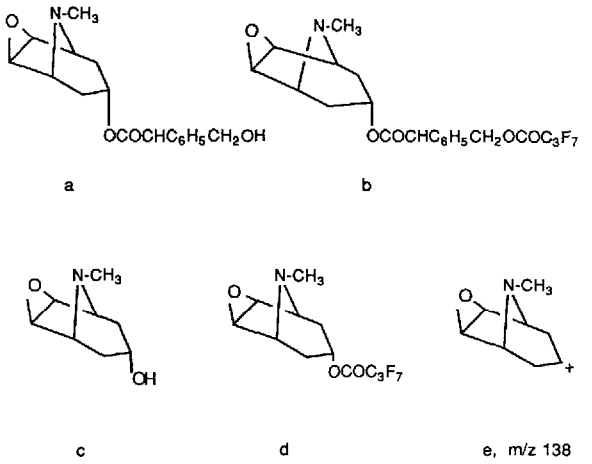



Fig. 1. Structures of scopolamine (a), heptafluorobutyrylscopolamine (b), scopine (c), heptafluorobutyrylscopine (d) and the *m/z* 138 ion (e).

because of the time-consuming nature and poor sensitivity of existing assays for scopolamine.

Among the most sensitive analytical methods for scopolamine are radioreceptor [4], gas chromatographic–mass spectrometric (GC–MS) [5] and high-performance liquid chromatographic (HPLC) [6] assays. They require preliminary chromatographic purification or acidic extractions prior to derivatization. Because of these limitations, we thought it important to develop a simple and direct assay for scopolamine with sufficient sensitivity to measure its pharmacokinetics in rodents or human subjects. We report here an improved GC–MS assay, using electron-impact detection, that is suitable for quantitation of scopolamine in various biological tissues.

## EXPERIMENTAL

### *Materials*

Trideuterated scopolamine·HBr was obtained from MSD Isotopes (Montreal, Canada). Scopolamine·HBr and scopine·HCl were obtained from Sigma (St. Louis, MO, U.S.A.) and heptafluorobutyric anhydride was from Pierce (Rockford, IL, U.S.A.). All solvents were of commercial HPLC quality. Adult male Fischer-344 rats, three months old and approximately 240 g weight, were from Charles River Breeding Labs. (Wilmington, MA, U.S.A.).

### *Plasma extraction*

Plasma (0.5 ml) was spiked with the internal standard, trideuterated scopolamine·HBr (20–100 ng), treated with 0.1 *M* $Na_2CO_3$ (0.5 ml) and extracted

twice with diethyl ether (2.0 ml). Following centrifugation, the organic layer was separated.

*Brain extraction*

Brain tissue (0.5 g) was sonicated (Heat Systems-Ultrasonics, Farmingdale, NY, U.S.A.) and spiked with trideuterated scopolamine·HBr (100–200 ng), treated with 1 *M* $K_2CO_3$ (1.0 ml) and extracted twice with diethyl ether (2.0 ml). Following centrifugation the organic layer was separated and mixed with 0.2 *M* HCl (0.3 ml).

Initial studies demonstrated that scopolamine in the brain extract could not be derivatized in high yield, probably due to binding of scopolamine to co-extracted brain constituents. Hence, alkali hydrolysis of the extract was performed, leading to the conversion of scopolamine to scopine. After removing the organic solvent from the acidified solution (above), 5 *M* NaOH solution (0.5 ml) was added and the solution was heated at 60 °C for 20 min. The alkali solution then was diluted with water (1.0 ml), extracted with methylene chloride (2.5 ml), washed with water (1.0 ml) and dried on anhydrous $CaCl_2$.

*Derivatization*

After removal of the solvent, the extracted material (Fig. 1a or c) was converted to its heptafluorobutyryl derivative (Fig. 1b or d) by adding a 5% (v/v) solution of heptafluorobutyric anhydride in toluene (10–20 μl) at room temperature. After 5 min, 1–3 μl of the toluene solution was injected into the GC–MS system for quantitation.

*Chromatography*

Samples were injected on to a HP 5890 gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.), equipped with a J&W DB5 capillary column, 30 m×0.25 mm (Bodman Chemicals, Aston, PA, U.S.A.). Samples were injected with the splitless valve on for 1.5 min and were run with the oven temperature at 90 °C for 1 min, followed by a 30 °C/min increase up to 200 °C. The injector was set at a temperature of 250 °C and the detector line at 280 °C. Retention times of heptafluorobutyrylscopine and heptafluorobutyrylscopolamine were 5.7 and 5.9 min, respectively, at 35 kPa helium pressure on the top of the column.

*Mass spectrometry*

Electron-impact GC–MS measurements were carried out on a HP 5971A (Hewlett-Packard) mass-selective detector. The selected-ion monitor (SIM) device was set to monitor the ions at *m/z* 138 and 141.

*Animal studies*

Rats were injected intraperitoneally with scopolamine·HBr, 2.5 mg/kg body weight, in distilled water (1.0 ml/kg). At times between 5 and 240 min, two

animals per time point were killed. Blood was collected, centrifuged (10 000 *g*, 60 s), and plasma and brain were immediately stored at −70°C.

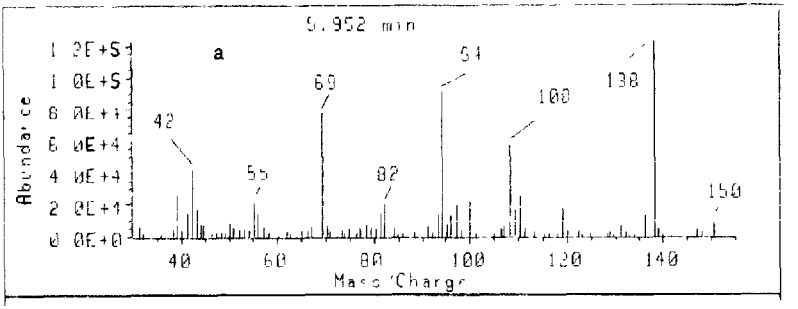
## RESULTS

### *Yields and stability*

Ether extraction of alkalinized blank rat plasma and brain spiked with scopolamine·HBr yielded a 90–95% extraction from plasma and 65–70% extraction from brain. The yield of derivatization of scopolamine was equivalent to that of scopine. Stability studies of scopolamine·HBr in plasma and in brain stored at −70°C demonstrated less than 3% loss after six weeks.

### *Mass spectrometry*

Electron-impact ionization of heptafluorobutyrylscopolamine did not produce a molecular ion peak at *m/z* 499. The mass spectrum showed abundant fragment ions at *m/z* 138, 108, 94 and 42 (Fig. 2a). All were shifted by three atomic units to *m/z* 141, 111, 97 and 45, respectively, when the trideuterated scopolamine derivative was assayed. The mass spectrum of heptafluorobutyr-



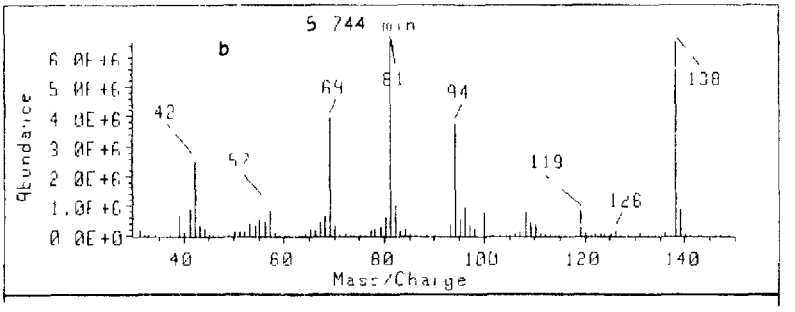




Fig. 2. Electron-impact mass spectra of heptafluorobutyrylscopolamine (a) and heptafluorobutyrylscopine (b).

ylscopine, as reported before [5], showed no molecular ion, but four fragment ions at *m/z* 138, 94, 81 and 69 were detected in high abundance (Fig. 2b). The first two peaks were shifted by three atomic units in the mass spectrum of trideuterated analogue.

*Detection in plasma*

The limit of linear detection in plasma, using SIM, was 1 ng per 0.5 ml. Two standard curves were constructed by spiking 0.5 ml blank plasma with 1–5 or 50–300 ng scopolamine·HBr, respectively, and with 20 or 100 ng trideuterated scopolamine·HBr, the internal standard. The regression coefficient for both standard curves was 0.999. Five replicate measurements of 50 ng per 0.5 ml, made on the same day, yielded results of 51.6 ng per 0.5 ml (coefficient of variation 4.3%). On three different days, concentrations were 51.2, 52.2 and 54.4 ng per 0.5 ml.

*Detection in brain*

The limit of linear detection in brain, using SIM, was 10 ng per 0.5 g. Two standard curves were constructed by spiking 0.5 g blank rat brain with 10–50 or 50–300 ng scopolamine·HBr and with 100 and 200 ng trideuterated scopolamine·HBr, respectively. The regression coefficient for both standard curves was 0.999. Five measurements on the same day at a concentration of 100 ng per 0.5 g yielded results of 98.6 ng per 0.5 g (coefficient of variation 6.8%). Heptafluorobutyrylscopolamine was stable in the reacting solution at room temperature for more than 8 h.

*Animal pharmacokinetics*

A single dose of scopolamine·HBr was administered intraperitoneally to rats, and plasma and brain concentrations were measured at time points between 5

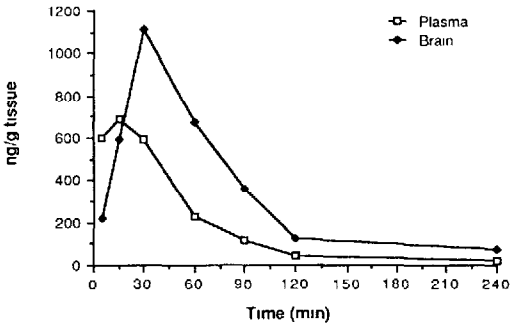



Fig. 3. Plasma and brain concentration versus time profile of scopolamine following intraperitoneal administration to rats of 2.5 mg/kg.

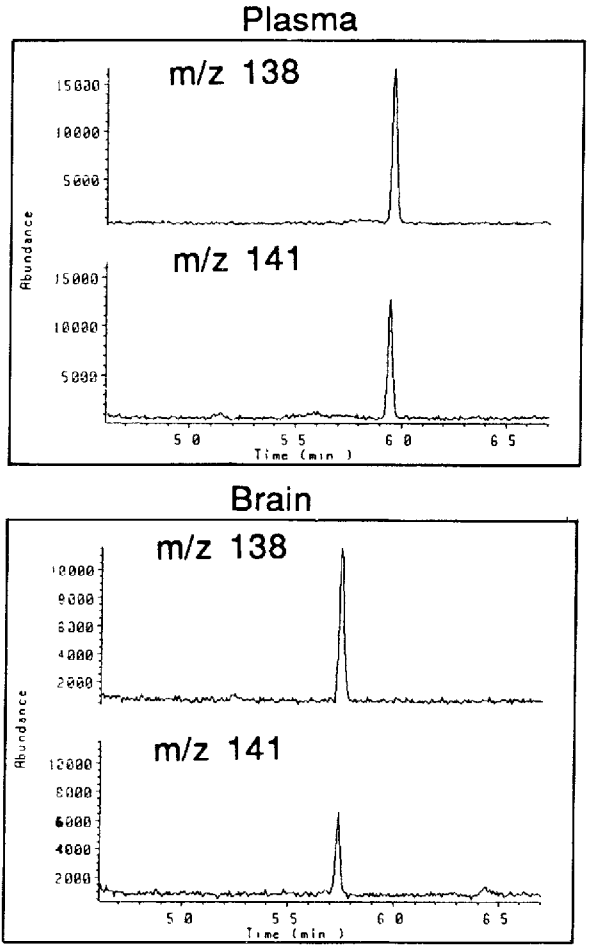



Fig. 4. Electron-impact SIM profiles obtained from plasma and brain taken 30 min after administration to rats of 2.5 mg/kg scopolamine·HBr intraperitoneally. Upper profiles (*m/z* 138) are due to scopolamine, whereas lower profiles (*m/z* 141) are due to trideuterated scopolamine added as internal standard (200 ng per 0.5 ml or 0.5 g).

and 240 min. The plasma and brain concentration versus time profile is shown in Fig. 3. Scopolamine could be detected in both plasma and brain at all time points, and brain scopolamine concentrations were larger than corresponding plasma levels at 30 min and later. A comparison of SIM profiles obtained from plasma and brain extracts, taken 30 min after a dose of 2.5 mg/kg scopolamine·HBr, is shown in Fig. 4. The internal standard concentration was 200 ng per 0.5 ml or 200 ng per 0.5 g, respectively. Although the extraction yield from brain was lower than from plasma, the brain concentration was two-fold larger at this time point.

## DISCUSSION

This report describes a sensitive GC–MS assay for scopolamine in biological tissue that is suitable for plasma and brain pharmacokinetic studies. For plasma, extracted scopolamine was directly derivatized with heptafluorobutyric anhydride and chromatographed. The resulting GC peak was monitored with a mass-selective detector in an electron-impact ionization mode. The four most abundant peaks in the fragmentation pattern were shifted in the mass spectrum of the trideuterated analogue by three atomic units, proving that their origin is the scopolamine derivative.

Due to the binding of scopolamine to brain constituents, extracted material from brain could not be directly derivatized. Extracted scopolamine from brain tissue therefore was quantified by a variation of a reported GC–MS method [5]. Brain extract containing scopolamine bound to other constituents was hydrolyzed with 5 *M* NaOH solution. Scopolamine then was converted to scopine and derivatized with heptafluorobutyric anhydride to heptafluorobutyrylscopine. Its mass spectrum, like that of scopolamine, contained a large peak at $m/z$ 138 which was shifted by three atomic units in the mass spectrum of the trideuterated analogue, the internal standard. This indirect method allows for contribution to measured levels of scopolamine in brain by metabolites in which the side-chain has been modified. However, no evidence of such metabolites was found in plasma samples.

A previous HPLC assay [6], designed for quantitation of scopolamine in pharmaceutical formulations, lacks sufficient sensitivity (20 ng per injection) for pharmacokinetic studies. The most promising radioreceptor assay reported [4] has high sensitivity (0.025 ng/ml) but is cumbersome, requiring sample extraction with reversed-phase octadecylsilane (Sep-Pak $C_{18}$). A reported GC–MS assay [5] (0.05 ng/ml) involves acidic extraction from methylene dichloride, which we have found to result in 10–20% exchange of deuterium atoms of the internal standard when its concentration is low (20 ng/ml). Our procedure, capable of detecting scopolamine·HBr in plasma and brain at concentrations of 1 ng per 0.5 ml or 10 ng per 0.5 g, respectively, uses a simple extraction of scopolamine and derivatization at room temperature with a minimum of reacting solution that can be injected directly into the GC–MS system, eliminating the need for hydrolysis of plasma scopolamine.

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