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Determination of scopolamine in human serum by gas chromatography–ion trap tandem mass spectrometry

R. Oertel*, K. Richter, U. Ebert, W. Kirch

Institute of Clinical Pharmacology, Medical Faculty Carl Gustav Carus, Technical University Dresden, Fiedlerstrasse 27, 01307 Dresden, Germany

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Abstract

The objective of this study was to develop a very sensitive and selective method for the determination of scopolamine in serum with a rapid and simple sample preparation. A capillary column gas chromatographic–ion trap tandem mass spectrometric technique has been applied. Scopolamine and the internal standard mexiletine were extracted from serum samples and cleaned up by using a single step liquid–liquid extraction. Derivatization was carried out using 2,2,2-trifluoro-N-methyl-N-trimethylsilylacetamide. The mass spectrometer was operated with positive ions in the selected reaction mode with chemical ionisation using methane. The sum of peak height of two daughter ions was used for quantification. The detection limit was 50 pg/ml in serum.

Keywords: Scopolamine

1. Introduction

Scopolamine is an anticholinergic alkaloid, chemically related to atropine [1,2]. Several high-performance liquid chromatographic (HPLC) methods have been developed for the analysis of scopolamine in plant and pharmaceutical samples or in urine [3–5]. The only approach that has been successfully applied to serum samples is radioreceptor assay [6,7]. A pharmacokinetic study has been based on gas chromatography–mass spectrometry (GC–MS) after hydrolysis and formation of heptafluorobutyryl derivatives [8]. For this method a 4-ml plasma sample and a complicated sample preparation were necessary to get a sensitivity of 50 pg/ml.

This paper describes the application of a capillary column gas chromatographic–ion trap tandem mass spectrometric (GC–MS–MS) technique with chemical ionisation (CI) to determine scopolamine in serum samples of volunteers. Derivatization of scopolamine and the internal standard mexiletine was carried out using 2,2,2-trifluoro-N-methyl-N-trimethylsilylacetamide (MSTFA) (Fig. 1a,b). MS–MS removes ions originating from impurities of the biological matrices that differ in m/z from the selected ion. Only a small sample volume and a simple sample preparation are necessary for measurement of scopolamine serum concentrations after a dosage of 0.4 mg.

*Corresponding author.

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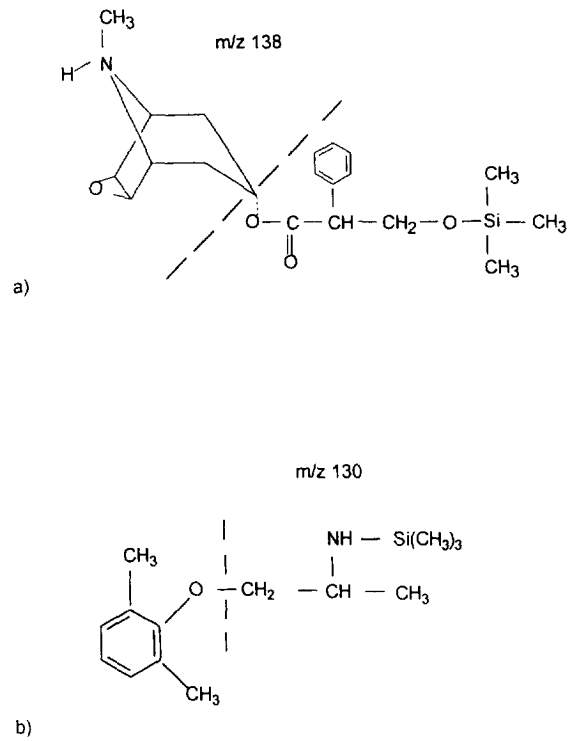

Fig. 1. Trimethylsilyl derivative of (a) scopolamine and (b) the internal standard mexiletine.

2. Experimental

2.1. Chemicals

Scopolamine hydrobromide DAB 10 was provided by Caesar und Kretz (Hilden, Germany). Mexiletine hydrochloride was obtained from Boehringer-Ingelheim (Ingelheim, Germany). MSTFA (for gas chromatography), dichloromethane LiChrosolv (for chromatography) and methanol LiChrosolv (for chromatography) were purchased from Merck (Darmstadt, Germany). Pure water (20 MΩ) was obtained using the ion-exchange system RS 40 E, SG Ionenaustauscher (Barsbüttel, Germany).

2.2. GC method

2.2.1. Apparatus and chromatographic conditions

The gas chromatograph was a Varian 3400 (Walnut Creek, CA, USA) with an autosampler A200S (Finigan-MAT, San Jose, CA, USA) and a septum programmable injector PTV (LAR, Berlin, Germany). Chromatography was performed via an injection on a Durabond 5 fused-silica capillary column, 15 m×0.25 mm I.D., film thickness of 0.1 μm (JW Scientific, Folsom, CA, USA). Carrier gas was helium with an inlet pressure of 70 kPa. The temperature programme of the column was as follows: 1 min isothermal at 80°C, ramped to 290°C at 30 °C/min, followed by an isothermal period of 0.1 min at 290°C. A 5-μl aliquot of the sample was injected at a temperature of 80°C with a split of 10 ml/min to remove the solvent. After 40 s the split was closed and the injector was ramped to 280°C at 300 °C/min. The temperature of the transfer line from the gas chromatograph to the ion trap was held at 250°C.

2.3. MS method

The samples were analysed using an ion trap detector Varian Saturn 3. The ion trap tandem mass spectrometer was operated with positive ions in the selected reaction mode with CI with methane. The peak height of daughter ions was used for quantification. The ion trap electrode assembly was held at 170°C.

It is possible to use different acquisition methods and different ion preparation parameters in the same chromatogram to optimize the conditions for several interesting substances. To prevent the detection of solvent the acquisition was started 3 min after injection. With a mass range from m/z 59 to 140 and a parent ion mass of m/z 130 in the resonant mode, the peak height of the daughter ion (m/z 73) of the internal standard was determined (product ion scanning). In the acquisition segments from 5 min to the end of the analyses a mass range from m/z 79 to 140 and a parent ion mass of m/z 138 in the non-resonant mode were used for determining the peak heights of the daughter ions (m/z 94 and m/z 110) of scopolamine. The acquisitions were obtained at different scan rates. Near the retention time of internal standard and scopolamine the scan time is minimal (~15 μs) to get enough points to define the peaks. For mass spectrometer parameters and for acquisition conditions see Table 1 and Table 2.

Table 1
Mass spectrometer parameters: acquisition segments in the mass spectrometer

Number	Run time (min)	Scan time (s)	Mass range (*m/z*)	Ion preparation method
0	0.0–3.0	delay		
1	3.0–3.3	1	59–140	Mex
2	3.3–3.8	0.17	59–140	Mex
3	3.8–5.0	1	59–140	Mex
4	5.0–6.0	1	79–140	Scop
5	6.0–6.5	0.16	79–140	Scop
6	6.5–7.0	1	79–140	Scop

2.4. Sample preparation

2.4.1. Extraction procedure

This method involves a single extraction of the compound from 1 ml serum in a 10-ml glass tube into 4 ml dichloromethane. The mixture was shaken for 20 s (Heidolph-Mixer). After centrifugation for 10 min at 2500 *g*, the organic phase was transferred into a 10-ml glass tube and evaporated to dryness at 80°C in a vacuum centrifuge (Jouan Evaporator centrifuge RC 10-20). Under these conditions, loss of water of the drug or hydrolysis to scopoline [8] was not observed.

2.4.2. Derivatization procedure

A 40-μl volume of MSTFA was added to the dried residue and mixed for 10 s. The sample was transferred into an autosampler vial and after standing for 1 h at 50°C, 5 μl of this mixture was injected onto the gas chromatographic system.

2.4.3. Standard solutions

Stock solutions of scopolamine and the internal standard mexiletine were prepared by dissolving the substances in methanol to a final concentration of 1 mg/ml. Working solutions were obtained by further dilution of the stock solutions with methanol.

Table 2
Mass spectrometer parameter: ion preparation methods

Method	Mex	Scop
Measurement	Internal standard	Scopolamine
Mass range (*m/z*)	59–140	79–140
Parent ion mass (*m/z*)	130	138
Mass isolation window (*m/z*)	3	3
Mode	Resonant	Non-resonant
Excitation time (ms)	20	20
Excitation amplitude (V)	0.5	35
Daughter ions (*m/z*)	70	94 and 110

2.4.4. Samples

Serum samples were obtained from healthy volunteers after a single dose of 0.4, 0.6 or 0.8 mg scopolamine by subcutaneous injection [10].

3. Results and discussion

3.1. Mass spectrometry

The mass spectrum of the trimethylsilyl (TMS) derivative of scopolamine shows the molecular ion $[M+H]^+$ at *m/z* 376 and a fragment ion at *m/z* 138 by methane CI. This fragment ion peak is about three times higher than that of the molecular ion. Other fragments can be neglected (Fig. 2). Using electron impact mode, a mass spectrum with

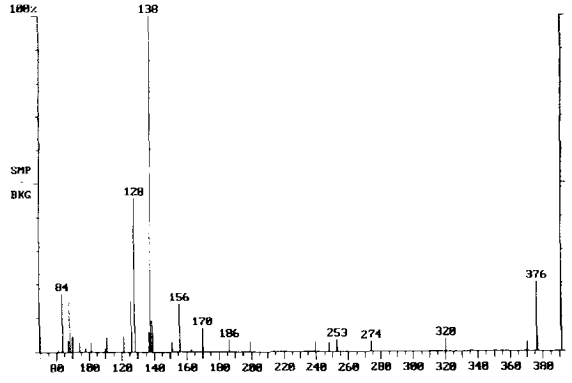

Fig. 2. CI mass spectrum of the trimethylsilyl derivative of scopolamine (1 ng in 40 μl MSTFA, injection volume 5 μl).

four characteristic fragment ions and the molecular ion with m/z values 94, 108, 138, 154 and 376, respectively, was obtained. The $[M+1]^+$ ion is a typical phenomena in ion trap electron ionisation (EI) spectra. The base peak in the EI spectrum was m/z 94 and the relative intensity of the $[M+1]^+$ ion was 15% (Fig. 3).

To improve the selectivity and the sensitivity of the method the ion trap detector should work in the MS–MS mode. In a biological matrix a lot of substances disturb the measurement. The majority of the matrix ions are ejected in the first isolation step, when the parent ion is isolated. For scopolamine the molecular ion or the fragment ion m/z 138 can be used as the parent ion. The fragment ions of the EI mass spectra were not fit for MS–MS. CI and the parent ion 138 was found as the most suitable method for determination of scopolamine in serum. The generation of specific daughter ions in the trap is the second isolation step to eject matrix ions. For the quantitative measurement of scopolamine, daughter ions with m/z 94 and m/z 110 were used (Fig. 4, Fig. 5). The fragment ion m/z 110 originates from the ion m/z 138, probably after loss of nitrogen and a methyl group and fragment ion m/z 94 after additional loss of oxygen and the structure of this ion is a ring $(C_7H_{10})^+$.

Using CI with methane, the mass spectrum of the trimethylsilyl derivative of the internal standard mexiletine gave the base peak at m/z 130 (Fig. 1b). This ion was selected to be the parent ion. For the quantitative measurement the daughter ion with m/z 73 (the trimethylsilyl group) was used (Fig. 5).

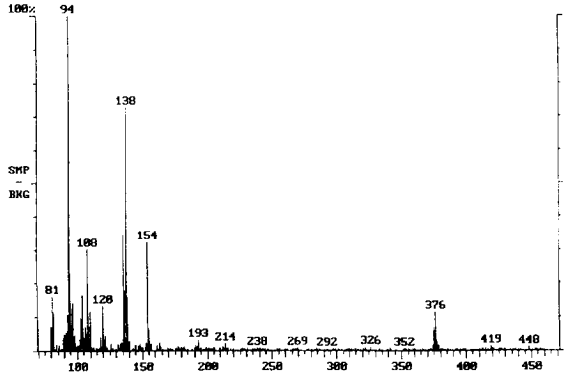

Fig. 3. EI mass spectrum of the trimethylsilyl derivative of scopolamine (4 ng in 40 μl MSTFA, injection volume 5 μl).

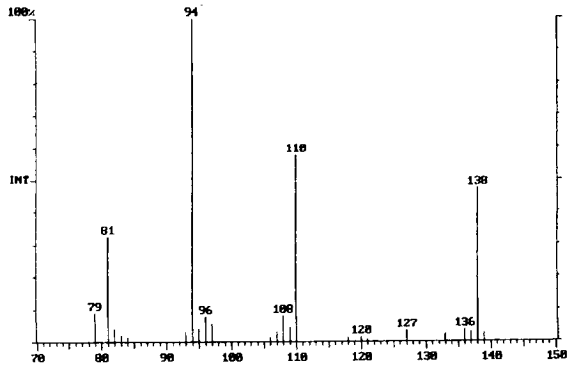

Fig. 4. MS–MS spectrum of scopolamine (1 ng in 40 μl MSTFA, injection volume 5 μl) having fragment ion (m/z 138) as the parent ion.

3.2. Chromatography

With the septum programmable injector it is possible to inject more than 1 μl of sample into the gas chromatograph to get a higher sensitivity. It is

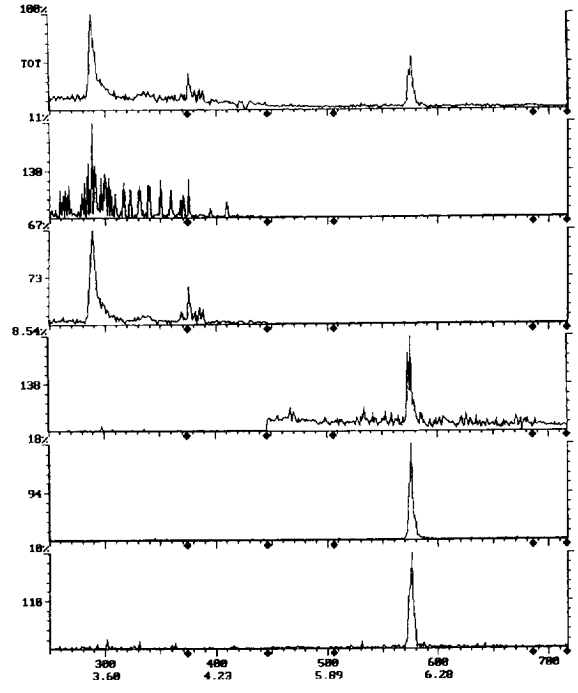

Fig. 5. Total-ion chromatogram and 5 characteristic single-ion chromatograms: parent (m/z 138) and two daughter ions (m/z 94 and 110) of the trimethylsilyl derivative of scopolamine and parent (m/z 130) and daughter ion (m/z 73) of the internal standard mexiletine (1 ng scopolamine and 100 ng mexiletine in 40 μl MSTFA, injection volume 5 μl).

necessary to optimize the injection: volume, rate, temperature time program, and split. However the possibilities of such optimization with autosamplers are limited, for example the maximum size of the syringe was 10 μl or the minimum rate of injection was 5 μl/s. For scopolamine a volume of 5 μl when injected at the lowest rate showed the best results.

For injection volumes of more than 1 μl, it is necessary to inject with split at temperatures below the boiling point. In our case the derivatization reagent (MSTFA) is also the solvent. It is well known that some derivatization reactions with MSTFA take place in the hot injection port [9]. When the samples are injected at 80°C the derivatization must be complete prior to injection.

Symmetrical peaks were observed for scopolamine and the internal standard. Typical chromatograms obtained from extracted serum samples are illustrated in Fig. 6a–c. The retention times of scopolamine and the internal standard were 6.5 and 3.6 min, respectively. The overall chromatographic run time was 7.0 min. The next injection followed after about 14 min.

3.3. Validation

The linearity and the precision were tested using spiked serum samples. The linearity of the method was confirmed in the range of 100 pg–4 ng/ml using a 1-ml sample. The precision of the method was assessed by determination of six concentrations in six independent series of samples as shown in Table 3. The correlation coefficient was 0.995 or higher. The lower limit of quantification, i.e., a coefficient of variation <20% for six repeated measurements, is 200 pg/ml. The minimum detectable concentrations, i.e., a signal-to-noise ratio of more than 3, was 50 pg/ml.

Human serum was collected from twelve healthy volunteers and screened for interference at the retention times of scopolamine and the internal standard. Fig. 6a shows typical chromatograms of a processed blank serum. It indicates that no endogenous compounds exist at the retention times of scopolamine or internal standard. A recovery of 80% of scopolamine from the serum matrix was found independent from the concentration within a range from 200 pg to 4 ng/ml. Scopolamine has been shown to be stable in serum maintained at −20°C for up to six months and at ambient temperature for

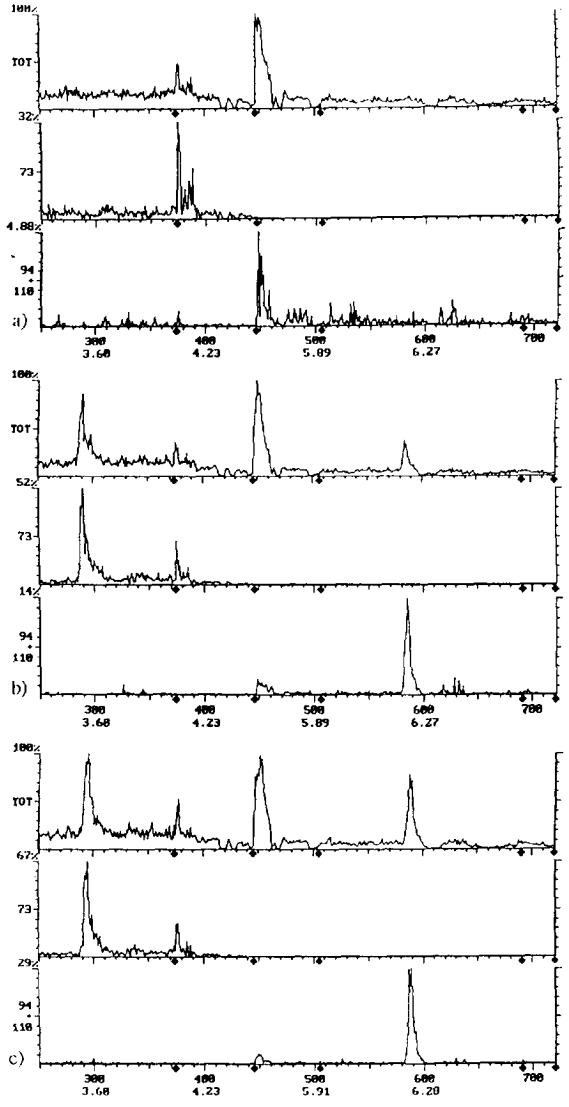

Fig. 6. Total-ion chromatograms and daughter-ion chromatograms (sum m/z 94 and m/z 110) of (a) blank serum, (b) blank serum spiked with scopolamine 0.5 ng/ml and (c) serum sample of a volunteer 30 min after a single dose of 0.4 mg scopolamine by subcutaneous injection.

Table 3
Precision of the analytical method to determine scopolamine

Concentration added (pg/ml)	Concentration recovered (mean±S.D.) (pg/ml)	Coefficient of variation (%)
200	219±25	11.3
500	499±45	8.9
1000	974±123	12.7
2000	2030±33	1.6
4000	4020±224	5.6

24 h. Stability has also been established through two freeze–thaw cycles for spiked serum samples.

4. Conclusion

This capillary column GC–MS–MS technique with CI was found to be selective and sensitive for the measurement of scopolamine in serum. Furthermore, the assay requires a simple sample preparation.

This assay allows the quantification of scopolamine serum levels for at least 8 h following a single 0.4-mg subcutaneous dose of scopolamine. To increase the sensitivity it is possible to increase the sample volume.

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