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Determination of scopolamine in human serum and microdialysis samples by liquid chromatography–tandem mass spectrometry

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Abstract

A liquid chromatographic-tandem mass spectrometric (LC–MS–MS) method with a rapid and simple sample preparation was developed for the determination of scopolamine in biological fluids. Scopolamine and the internal standard atropine in serum samples were extracted and cleaned up by using an automated solid phase extraction method. Microdialysis samples were directly injected into the LC–MS system. The mass spectrometer was operated in the multi reaction monitoring mode. A good linear response over the range of 20 pg/ml to 5 ng/ml was demonstrated. The accuracy for added scopolamine ranged from 95.0 to 104.0%. The lower limit of quantification was 20 pg/ml. This method is suitable for pharmacokinetic studies. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Scopolamine

1. Introduction

Scopolamine is an anticholinergic alkaloid, chemically related to atropine (Fig. 1) [1,2]. Several high performance liquid chromatographic (HPLC) methods were developed for the analysis of scopolamine in plants and pharmaceutical samples or in urine [3,4]. Pharmacokinetic studies have been based on gas chromatography–mass spectrometry (GC–MS) [5–8]. Using these methods 1 or 4 ml of plasma and complicated sample preparation with liquid extraction and derivatisation were necessary to achieve a sensitivity of 50 pg/ml.

Microdialysis sampling is a suitable tool for measuring drug concentrations in human subcutaneous tissues. Microdialysis is readily applicable,

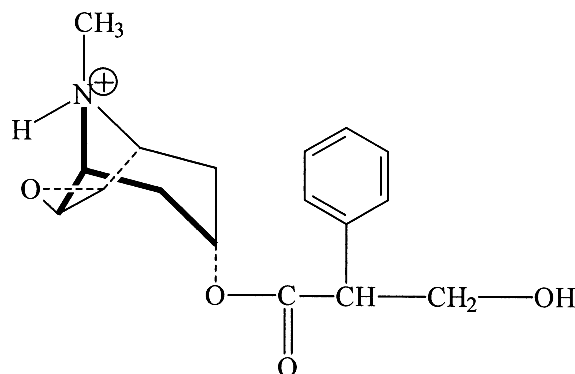

Fig. 1. Structural formulae of scopolamine.

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relatively non-invasive, and reproducible. This technique is a valuable addition for pharmacokinetic characterisation of selected drugs [9,10]. Capillary zone electrophoresis, coupled to UV and interfaced with electrospray ionisation mass spectrometry, is described for the analysis of scopolamine in plant material [11].

This paper describes a liquid chromatographic–tandem mass spectrometric (LC–MS–MS) method with a rapid, simple and automated solid phase extraction (SPE) sample preparation to determine scopolamine in serum samples of volunteers, requiring smaller serum volumes and enabling a lower limit of quantification. Less than 60 μl of the microdialysis from these volunteers samples were available. Using the developed method, those microdialysis samples could also be analysed.

2. Experimental

2.1. Chemicals

Scopolamine hydrobromide DAB 10 was provided by Caesar u. Kretz (Hilden, Germany). Atropine was obtained from Synopharm (Basel, Switzerland). Acetonitrile LiChrosolv (for chromatography), methanol LiChrosolv (for chromatography), formic acid (p.a.) and ammonium acetate (p.a.) were purchased from Merck (Darmstadt, Germany). Ringer solution was obtained from Serumwerk Bernburg (Bernburg, Germany). Pure water (18 MΩ) was obtained using an ion-exchange system RS 40 E, SG Ionenaustauscher (Barsbüttel, Germany).

2.2. LC–MS–MS analysis

2.2.1. Apparatus and chromatographic conditions

The LC–MS–MS system used was a API 3000 (PESCIEX, Concord, ON, Canada) equipped with a turbo ion spray interface. The capillary voltage was 5800 V (positive ion mode). Full scan mass spectra were acquired by continual infusion of standard solutions (concentration: 100 ng/ml with 10 μl/min) and by scanning Q1 from *m/z* 100–350. The product ion mass spectra were obtained by choosing the molecular ions as the precursor ions, scanning Q2 from *m/z* 50–310 and optimised with the help of the program Autotune.

The multiple reaction monitoring (MRM) was performed by monitoring the transitions between *m/z* 304 and 138 (collision energy −42 eV, dwell time 395 ms) and between *m/z* 304 and 156 (collision energy −34 eV, dwell time 395 ms) for scopolamine and by monitoring the transitions between *m/z* 290 and 124 (collision energy −42 eV, dwell time 195 ms) for atropine.

The HPLC equipment consisted of two micro pumps and an autosampler (both series 200, Perkin Elmer, Norwalk, CT, USA). The chromatographic separation was performed on a Purospher STAR RP-18e column (55 mm×2 mm, 3 μm; Merck). The following mobile phase gradient was applied with solvent A (5:95:0.2, v/v/v) and solvent B (95:5:0.2, v/v/v) of a mixture of acetonitrile–ammonium acetate in water (0.002 mol/l)–formic acid (see Table 1) at a flow rate of 0.3 ml/min. The temperature in the turbo-ion-spray source was 400°C at a nitrogen gas flow of 8 ml/min.

Peak areas and the calibration curve were obtained using the TurboQuan program (PESCIEX, Concord, ON, Canada).

Table 1

Time (min)	0	0.1	1.1	2.1	2.2	9.0
A (%)	100	100	50	50	100	100
B (%)	0	0	50	50	0	0

2.3. Sample preparation

2.3.1. Solid phase extraction

Scopolamine and the internal standard atropine in serum samples were extracted and cleaned up by using an automated solid phase extraction method with Oasis cartridges HLB 1 cc (Waters) in an ASPEC XL Sample Processor (Gilson). The sample volume of 0.2 ml of serum, spiked with 2 ng of the I.S. dissolved in 10 μl of methanol, was diluted with 0.2 ml of phosphate buffer (0.05 mol/l, pH 8). The mixture was shaken for 20 s (Heidolph-Mixer). All

liquids and air were pressed through the cartridges. This is in contrast to the most other tools, which draw the liquids through the cartridges.

2.3.2. Extraction procedure

- Condition: 1 ml of methanol and 1 ml of water, pushing with 1 ml of air
- Load: mixture of 0.2 ml of serum and 0.2 ml of phosphate buffer, pushing with 1 ml of air
- Wash: 2 ml of water and 2 ml of 10% methanol in water, pushing with 1 ml of air
- Elute: 0.4 ml of methanol, pushing with 1 ml of air

Eluates were evaporated to dryness at 70°C in an air stream with a Techne DRI Block SC-3 (thermo-DUX, Wertheim, Germany) and redissolved in 100 μl of water. Microdialysis samples were injected directly into the LC–MS–MS system without an internal standard.

2.3.3. Standard solutions

Stock solutions of scopolamine and the internal standard atropine 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.

2.4. Samples

Serum samples were obtained from healthy volunteers receiving a single dose of 0.5 mg of scopolamine by intravenous injection.

Microdialysis is based on the sampling of analytes from the extracellular space by diffusion through a semipermeable membrane [9,10]. This process is accomplished in vivo by use of a microdialysis probe that is constantly perfused with a physiologic solution (ringer solution) at a low flow rate (3 μl/min). Once the probe is implanted into the abdominal subcutaneous tissue, substances are filtered by diffusion from the extracellular fluid into the perfusion medium. Samples were collected in intervals of 20 min. Less than 60 μl of the microdialysis samples were available [12–14].

3. Results

3.1. Mass spectrometry

The mass spectra of scopolamine and atropine are shown in Fig. 2. Each spectrum revealed a base peak at m/z 304 and 290, respectively, corresponding to the molecular ions $(M+H)^+$. The product ion mass spectra were obtained by choosing the molecular

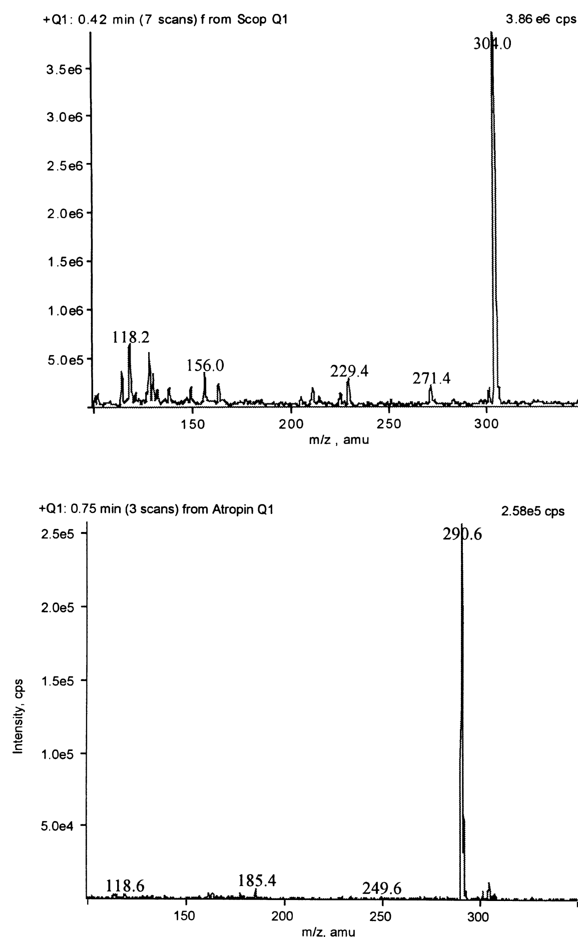

Fig. 2. (a) Mass spectrum (Q1) of scopolamine (100 ng/ml, flow rate 10 μl/min). (b) Mass spectrum (Q1) of atropine (100 ng/ml, flow rate 10 μl/min).

ions as the precursor ions. The fragment ions observed at *m*/*z* 138 and 156 for scopolamine and at *m*/*z* 124 for atropine are believed to result from a rearrangement of the tropan ring and the carboxyl group. The difference between the structure of scopolamine and atropine is the epoxy group on the tropan ring of scopolamine. This group is the reason for the different fragmentation of both substances. The stability of the fragment *m*/*z* 156 of scopolamine is probably conditioned by interactions between the epoxy and the carboxy group. The equivalent fragment *m*/*z* 142 of atropine has a very low intensity. Intensities of other fragments are negligible (Fig. 3).

3.2. Chromatography

In addition to the impact on LC separation, changes in the mobile phase can alter the sensitivity by influencing the ionisation efficiency of the analytes on MS. Working in the MRM-mode a complete separation of analytes and background are not necessary. Therefore, we need to optimise the mobile phase to obtain high sensitivity and short analysis time. A mixture of acetonitrile, ammonium acetate in water (0.002 mol/l) and formic acid gave a high sensitivity of scopolamine. Using an acetonitrile gradient, small and symmetrical peaks were observed for scopolamine and the internal standard. To exclude interferences from the biological matrix, chromatograms of the transitions between *m*/*z* 304 and *m*/*z* 138 and between *m*/*z* 304 and *m*/*z* 156 were controlled separately. No interferences and a low background noise were found. The best sensitivity was achieved by monitoring the sum of both fragment ions. For quantification of scopolamine concentration in serum the ratio of peak area of both fragments of scopolamine and peak area of atropine was used. For microdialysis samples peak areas of both fragment ions of scopolamine were used for concentration determination. These samples were measured directly without sample preparation. MS–MS does not respond to ions originating from impurities of the biological matrices that differ in *m*/*z* from the selected ion. The retention times of scopolamine and the internal standard were 3.3 and 3.6 min, respectively. The overall chromatographic run time was 4 min. The next injection followed after about 10 min.

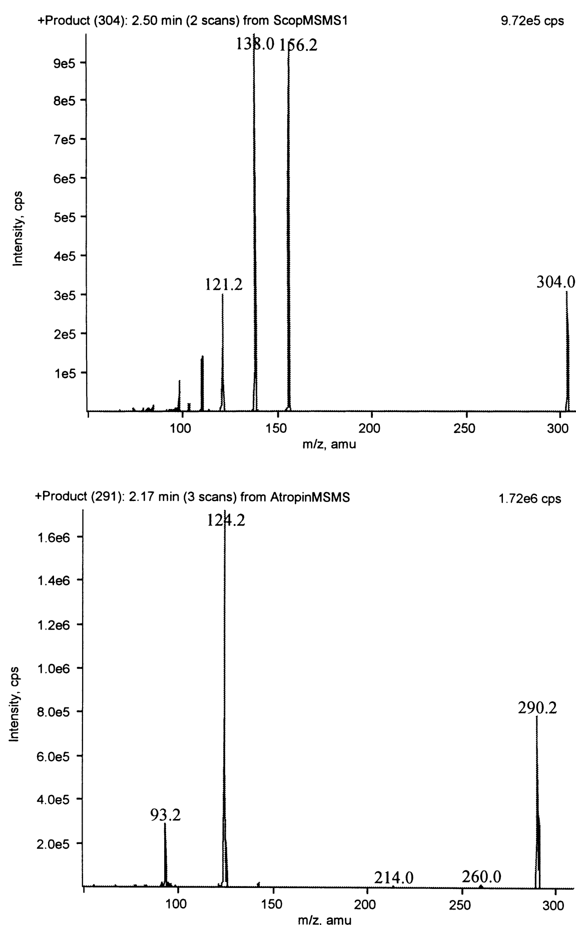

Fig. 3. (a) Product ion mass spectrum of scopolamine (100 ng/ml, flow rate 10 μl/min) molecular ion (m/z 304) was chosen as the precursor ion. (b) Product ion mass spectrum (Q1) of atropine (100 ng/ml, flow rate 10 μl/min) molecular ion (m/z 290) was chosen as the precursor ion.

3.3. Calibration graph

The calibration graph for scopolamine was generated from MRM of increasing amounts of scopolamine standard, and blank serum samples were spiked with constant levels of atropine as an internal standard. A linear calibration graph was constructed using least-squares regression of quan-

tities versus peak area ratio. A good linear response over the range of 20 pg/ml to 5 ng/ml serum was demonstrated. Samples with a higher concentration of scopolamine were diluted. The correlation coefficient of regression lines was 0.9968 or higher (Table 2). For microdialysis samples the linearity of the method was confirmed in the range of 50 pg/ml to 10 ng/ml.

3.4. Reproducibility

The precision and accuracy of the method was assessed by determination of seven concentrations in six independent series of spiked serum samples as shown in Table 2. The accuracy for added scopolamine ranged from 93.0 to 104.0%. The coefficient of variation ranged from 2.9 to 10.9%. The lower limit of quantification, i.e., a coefficient of variation <20% for six repeated measurements, is 20 pg/ml. Typical chromatograms obtained from extracted serum samples are illustrated in Fig. 4. Recovery of 51% from the serum matrix was found independent of the concentration. Similar results were obtained for microdialysis samples: accuracy ranged from 92.4 to 105.1%, precision from 3.4% to 12.9% and the lower limit of quantification is 50 pg/ml. The recovery was 100%. Day-to-day precision data were obtained over a period of 3 working days by taking aliquots of serum with 60 pg/ml, 300 pg/ml and 2100 pg/ml scopolamine, respectively, and processing them daily. Adequate coefficients of variation were found: 14.1% (lowest concentration), 9.4% (medium concentration) and 5.9% (highest concentration). For typical chromatograms see Fig. 5

3.5. Application to human volunteers

Scopolamine concentrations in human serum and microdialysis samples from six healthy male volunteers were studied over a period of 6 h. Scopolamine was detected in all samples in a range of 64 pg/ml to 13.3 ng/ml in serum samples and in a range of 98 pg/ml to 3020 pg/ml (3 ng/ml) in microdialysis samples.

4. Discussion

Liquid chromatography–tandem mass spectrometry is a powerful technique for highly specific and quantitative measurement of extremely low levels of analytes in biological matrices [15,16]. There have also been two reports on the use of a highly sensitive and specific, but rather complicated gas chromatography–mass spectrometry technique, to measure scopolamine in serum [5,8].

In this study a microanalytical method of scopolamine determination using LC–MS–MS after simple solid phase extraction has been developed. Only the small sample volume of 0.2 ml of serum was necessary to achieve a limit of quantification of 20 pg/ml.

From the microdialysis samples, less than 60 μl were available for analyses. Sample preparation procedures with such small volumes are complicated [13,14,17]. Best results were obtained with direct injection of samples into the LC–MS–MS system. The microdialysis samples contain non-volatile sodium and chloride ions. To avoid a loss of sensitivity, the curtain plate in the mass spectrometer should

Table 2
Precision and accuracy of the analytical method to determine scopolamine

Concentration added (pg/ml)	Concentration recovered mean±SD (pg/ml)	Coefficient of variation (%)	Accuracy (%)
20	18.6±1.4	7.6	93.0
50	48.8±5.3	10.9	97.5
100	104.0±6.1	5.9	104.0
200	205.0±12.7	6.2	102.3
500	5200.0±27.0	5.1	104.1
1000	1005.0±68.0	6.8	100.5
2000	1968.0±57.0	2.9	98.4

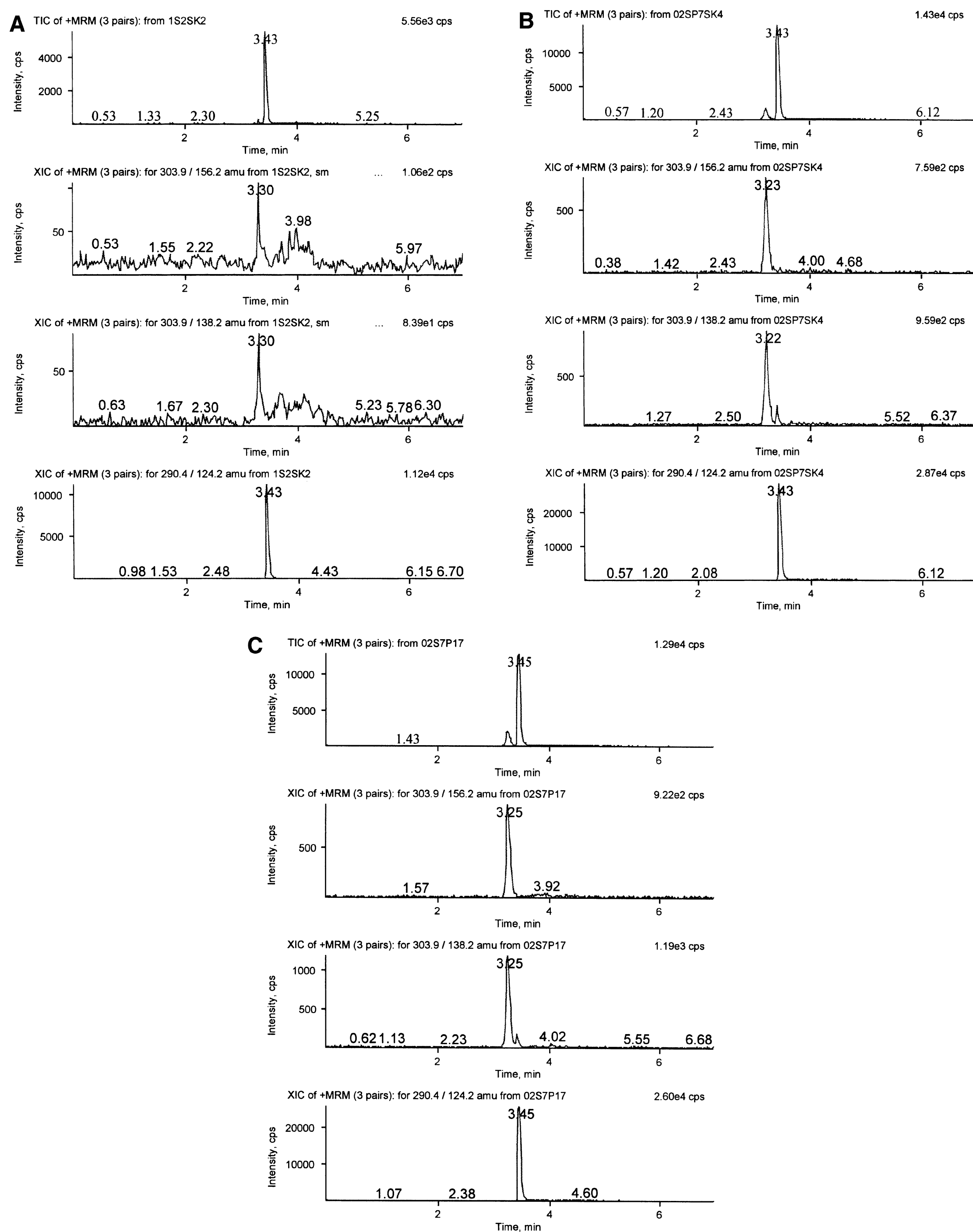

Fig. 4. TIC and MRM chromatograms of scopolamine extracted from serum. MRM was performed by monitoring the transitions between m/z 304 and m/z 156 and between m/z 304 and m/z 138 for scopolamine and between m/z 290 and m/z 124 for atropine. (a) blank serum spiked with atropine, (b) blank serum spiked with 50 pg/ml scopolamine and with atropine, (c) serum sample of a volunteer 240 min after a single dose of 0.5 mg scopolamine by intravenous injection.

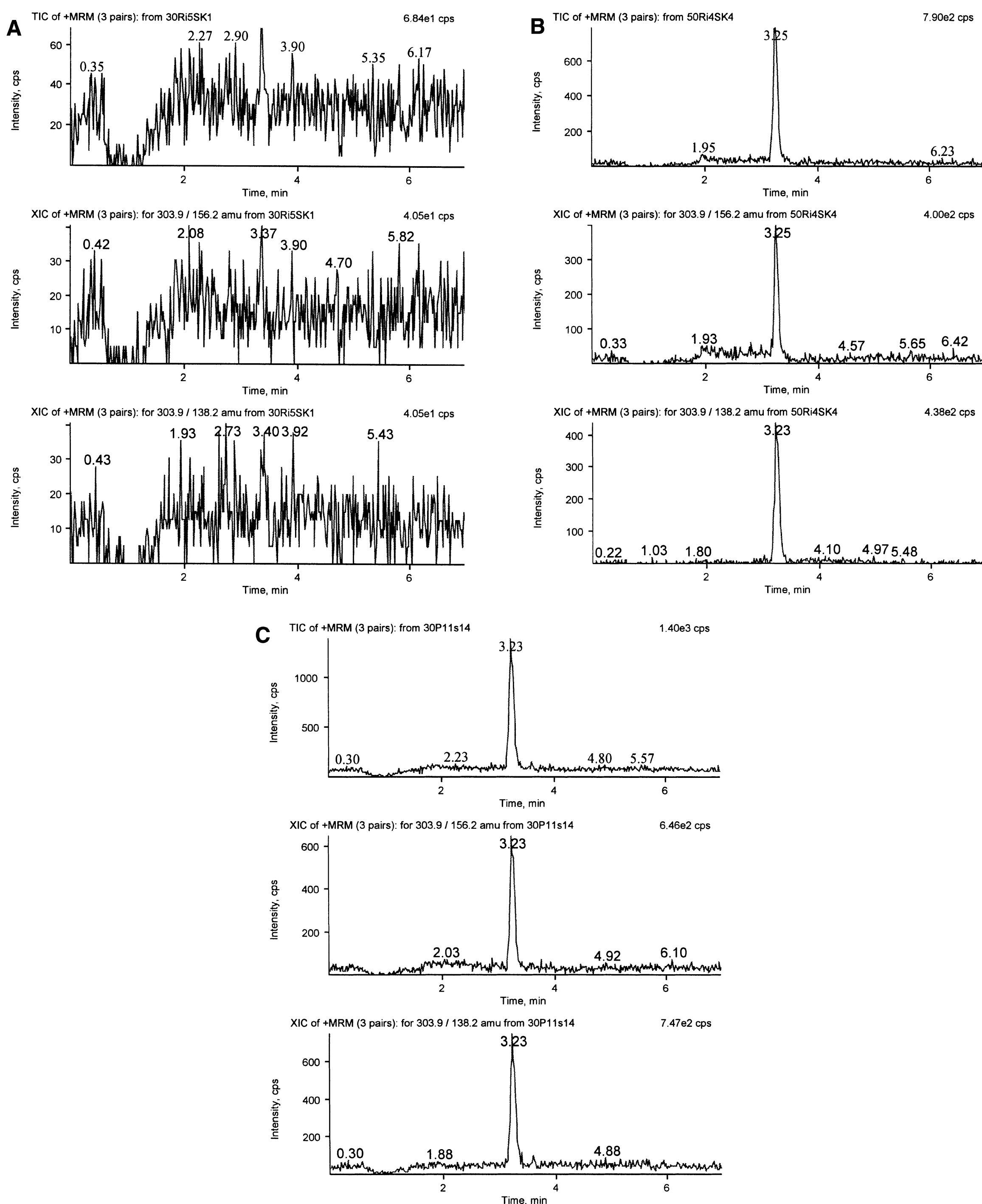

Fig. 5. TIC and MRM chromatograms of scopolamine in microdialysis samples. MRM was performed by monitoring the transitions between m/z 304 and m/z 156 and between m/z 304 and m/z 138 for scopolamine. (a) blank microdialysis sample, (b) blank microdialysis sample spiked with 100 pg/ml scopolamine, (c) microdialysis sample of a volunteer 240 min after a single dose of 0.5 mg scopolamine by intravenous injection.

be cleaned after approximately each 100 injections of 50 μl of physiological solution.

5. Conclusion

LC–MS–MS is the most sensitive method for quantitating scopolamine in serum and the only method for quantitating scopolamine in microdialysis samples. Furthermore, the assay requires only an automatic simple sample preparation. To increase the sensitivity it is possible to increase the sample volume. This method is suitable for pharmacokinetic studies [6].

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