Journal of Chromatographic Science, Vol. 46, January 2008

# Liquid Chromatography–Electrospray Ionization Ion Trap Mass Spectrometry for Analysis of in Vivo and in Vitro Metabolites of Scopolamine in Rats

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## Abstract

**In vivo and in vitro metabolism of scopolamine is investigated using a highly specific and sensitive liquid chromatography–mass spectrometry (LC–MS$^n$) method. Feces, urine, and plasma samples are collected individually after ingestion of 55 mg/kg scopolamine by healthy rats. Rat feces and urine samples are cleaned up by a liquid–liquid extraction and a solid-phase extraction procedure (C18 cartridges), respectively. Methanol is added to rat plasma samples to precipitate plasma proteins. Scopolamine is incubated with homogenized liver and intestinal flora of rats in vitro, respectively. The metabolites in the incubating solution are extracted with ethyl acetate. Then these pretreated samples are injected into a reversed-phase C18 column with mobile phase of methanol–ammonium acetate (2mM, adjusted to pH 3.5 with formic acid) (70:30, v/v) and detected by an on-line MS$^n$ system. Identification and structural elucidation of the metabolites are performed by comparing their changes in molecular masses ($\Delta M$), retention-times and full scan MS$^n$ spectra with those of the parent drug. The results reveal that at least 8 metabolites (norscopine, scopine, tropic acid, aponorscopolamine, aposcopolamine, norscopolamine, hydroxyscopolamine, and hydroxyscopolamine *N*-oxide) and the parent drug exist in feces after administering 55 mg/kg scopolamine to healthy rats. Three new metabolites (tetrahydroxyscopolamine, trihydroxy-methoxyscopolamine, and dihydroxy-dimethoxyscopolamine) are identified in rat urine. Seven metabolites (norscopine, scopine, tropic acid, aponorscopolamine, aposcopolamine, norscopolamine, and hydroxyscopolamine) and the parent drug are detected in rat plasma. Only 1 hydrolyzed metabolite (scopine) is found in the rat intestinal flora incubation mixture, and 2 metabolites (aposcopolamine and norscopolamine) are identified in the homogenized liver incubation mixture.**

## Introduction

Drug metabolism experimentation has played an important role in drug discovery, design, and clinical application. Therefore, fast and efficient ways to provide accurate information about drug metabolism on the target compounds and their major metabolites are required (1,2). In the past, gas chromatography (GC) with electron capture detection or nitrogen phosphorus detection and high-performance liquid chromatography (HPLC) with UV spectrophotometric detection, fluorescence detection, or electrochemical detection, were the main methods for detecting drugs and their major metabolites in vivo. But these technologies cannot provide high enough sensitivity, specificity, and molecular structural information for the qualitative assay of drugs and their metabolites. The coupled GC–mass spectrometric (MS) technology can overcome these insufficiencies, but it often requires a time-consuming process of deriving compounds (3,4). Therefore, this method is not suitable for the detection of thermolabile, highly polar, or non-volatile metabolites, either.

Because the introduction of atmospheric pressure ionization interfaces, liquid chromatography–mass spectrometry (LC–MS) has been increasingly used to determine drugs and their metabolites for pre-clinical and clinical studies (5,6). The LC–MS system allows for the analyses of thermolabile, highly polar, and non-volatile metabolites, owing to its soft-ionization technique and high sensitivity. The target compounds can be directly determined in mixtures without complicated sample preparation or derivatization. Compared with LC–MS, LC–MS$^n$ can give us additional structural information and high specificity for qualitative analysis at trace levels. It has been proven to be a powerful approach for the metabolic identification of drugs (7–9). Therefore, the LC–MS$^n$ technique is frequently the initial choice for metabolite detection and identification. Analytes can be determined quantitatively or qualitatively in mixtures by LC–MS(MS$^n$) using scheduled ion monitoring (SIM), full scan MS$^2$, or selected reaction monitoring (SRM) modes even without good chromatographic separation, as long as the compounds have different molecular mass, because only special molecular ions are selected to be detected, and other compounds can be filtered. Isomers with different product ions can be detected in SRM mode even without good chromatographic separation. Structural elucidation of drug metabolites using LC–MS$^n$ is based on the premise that metabolites retain the substructures of the parent drug molecule. MS–MS product ion spectrum of

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each metabolite provides detailed substructural information of its structure. So, using the product ion spectrum of parent drug as a substructural template, metabolites presented in crude mixtures may be rapidly identified and detected based on their changes in molecular masses (ΔM) and spectral patterns of product ions, even without standards for each metabolite (10–12).

Scopolamine is a kind of tropane alkaloid separated from various solanaceous species (13), such as the roots of Chinese traditional medicine *Anisodus tanguticus* (Maxim.) Pascher. Scopolamine has widespread physiological activities such as spasmolytic, anaesthetic, acesodyne, and ophthalmic effects (14,15). In recent years, more and more pharmacological activities of scopolamine have been investigated and widely noticed. Compared with the comprehensive investigations into its therapeutic purpose, the study of its metabolism in vivo or in vitro is limited, although the metabolic study of scopolamine plays an important role in the development of new drugs and their clinical applications.

Some analytical assays have been developed for the quantitation of scopolamine in plants and pharmaceutical samples or in urine, based on capillary electrophoresis–diode array-electrospray–MS (16) and HPLC (17–19). Also, pharmacokinetic studies have been performed by GC–MS (20–22) and LC–$MS^n$ (23). However, metabolic studies of scopolamine in vivo have not been reported except in three papers (24–26). Werner and Schmidt (24) described the formation of metabolites such as 6-hydroxyatropine, scopine, and scopolamine glucuronide. Wada and Yamada et al. (25,26) found three major metabolites (*p*-hydroxyscopolamine, *m*-hydroxyscopolamine, and *p*-hydroxy-m-methoxyscopolamine) and the unchanged drug in rat by the combination of multi-approaches such as thin-layer chromatography, GC, GC–MS, and nuclear magnetic resonance. Because scopolamine and its metabolites are highly polar, thermolabile, and easy to undergo pyrolysis in the injector block of a GC, the GC or GC–MS method used is not satisfactory in the analysis of metabolism of scopolamine. In addition, the urine samples were prepared using 7% HCl at 100°C, which result in many metabolites decomposing.

We applied the LC–$MS^n$ technique to identify the metabolites of scopolamine in the rat (27). LC–$MS^n$ provides molecular mass and structural information depending upon fragmentation patterns. It permits direct analysis of intact polar non-volatile conjugates without derivatization and/or hydrolysis. 18 metabolites were found in rat urine. The major metabolic pathway of scopolamine in rat was hydrolysis, demethylation, dehydration, hydroxylation, and sulfate and glucuronide conjugate.

In order to comprehensively study the metabolism, this work presents the metabolism of scopolamine in rat feces, urine, and plasma after administration. The present study also involves the incubation of scopolamine with intestinal flora and homogenized liver in order to clarify its in vivo and in vitro metabolic pathways. Three new metabolites (tetrahydroxyscopolamine, trihydroxy-methoxyscopolamine, and dihydroxy-dimethoxyscopolamine) were found in rat urine after administering 55 mg/kg scopolamine. The parent drug and its metabolites 7 and 8 (described later) were found in rat feces and plasma, respectively. Only metabolites 1 and 2 of scopolamine were identified in rat intestinal flora and homogenized liver incubation mixtures in vitro, respectively. These metabolites were detected for the first time in rat feces, plasma, intestinal flora, and homogenized liver incubation solutions, which will be useful for future studies involving scopolamine, such as clinical therapy.

## Experimental

### Reagents and chemicals

Scopolamine hydrobromide was purchased from Sigma (St. Louis, MO). Methanol was of HPLC grade (Fisher Chemical Co., Inc., Los Angeles CA); water was deionized and double distilled; all other reagents were of analytical reagent grade.

### Apparatus

LC–MS and LC–$MS^n$ experiments were performed on an LCQ Duo quadrupole ion trap MS (ThermoFinnigan Corp, San Jose, CA) with a TSP4000 HPLC pump and a TSP AS3000 autosampler. The software Xcalibur version 1.2 (Finnigan) was applied for system operation and data collection. A high-speed desk centrifuge (TGL-16C, Shanghai Anting Scientific Instrument Factory, Shanghai, China) was used to centrifuge the samples. Rat urine samples were extracted on a C18 solid-phase extraction cartridge (3 mL/200 mg, AccuBond$^{II}$, Agilent, Palo Alto, CA). The intestinal incubation experiments were carried out in anaerobical incubation bags (AnaeroPouch-Anaero 08G05A-23, Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan) using anaerobical generating bags (Mitsubishi Gas Chemical Company, Inc.).

### Sample preparation

*In vivo samples*

Five wistar rats (180 ± 5 g, Hubei Research Center of Laboratory Animals, Hubei China) were housed in metabolic cages for the collection of feces, urine, and plasma. The rats were fasted for 24 h but with access to water, and then they were administered 55 mg/kg oral gavage doses of scopolamine. Feces and urine were collected individually during the time period 0–24 h. The samples were stored at –20°C until analysis. Heparinized blood samples of 200 µL were collected at 0.24, 0.75, 2, 9, 18, and 24 h from the ophthalmic veins of the rats by sterile capillary tube, then shaken and centrifuged at 2000 × *g* for 10 min. The supernatants were decanted, and immediately frozen at –20°C until analysis.

The feces samples were homogenized with water. An aliquot of 500 µL feces homogenate was extracted twice with 2 mL of ethyl acetate after adding 50 µL of 0.001% $Na_2CO_3$ solution. The supernatant ethyl acetate layers were decanted and pooled and evaporated at 37°C under nitrogen. The residue was redissolved in 500 µL of mobile phase and filtered through 0.45 µm film and an aliquot of 10 µL was used for LC–$MS^n$ analyses.

An aliquot of 1 mL of mixed 0–24 h urine samples was loaded onto a C18 solid-phase extraction cartridge which was preconditioned with 2 mL of methanol and 1 mL of water. Then, the SPE cartridge was washed with 2 mL of water, and the analytes were eluted with 1 mL of methanol. The elution solutions were filtered through 0.45 µm film and an aliquot of 10 µL was used for LC–MS$^n$ analyses.

An aliquot of 200 µL of the plasma samples was added to 300 µL of methanol to precipitate plasma proteins, and then centrifuged at 2000 × *g* for 10 min. The supernatant was filtered through 0.45 µm film and an aliquot of 10 µL was used for LC–MS$^n$ analyses.

*In vitro samples*

*Preparation of anaerobical cultural solutions (28).* Solution A, 37.5 mL (0.78% $K_2HPO_4$), solution B, 37.5 mL [0.47% $KH_2PO_4$, 1.18% NaCl, 1.2% $(NH_4)_2SO_4$, 0.12%$CaCl_2$, 0.25%$MgSO_4 \cdot H_2O$], solution C, 50 mL (8% $Na_2CO_3$), 0.5 g L-cysteine, 2 mL 25% L-ascorbic acid, 1 g eurythrol, 1 g tryptone, and 1 g nutrient agar were mixed together and diluted to 1 L with distilled water. HCl (2M) was used to adjust the mixture solution to pH 7.5~8.0.

*Metabolism in intestinal bacteria*

The fresh intestinal contents were obtained from wistar rat (200 g). Samples were homogenized with a glass rod in anaerobical cultural solution as the ratio of 0.5 g:1.5 mL immediately. Then, the homogenates were filtrated using gauze. Scopolamine was added into an intestinal flora cultural solution in culture dishes to a final concentration of 50 µg/mL. The culture dishes were put in anaerobical incubation bags. The anaerobical generating bags were opened, and put into anaerobical incubation bags immediately, then sealed. Incubations were carried out in a shaking water-bath at 37ºC anaerobically. The incubation was continued for 4 and 24 h, terminated, and extracted (twice) with ethyl acetate. The organic extracts were combined and evaporated at 37°C under nitrogen. The residues were reconstituted in 0.6 mL of mobile phase and centrifuged at 13000 × *g* for 10 min. The supernatant (0.5 mL) was used for LC–MS$^n$ analyses.

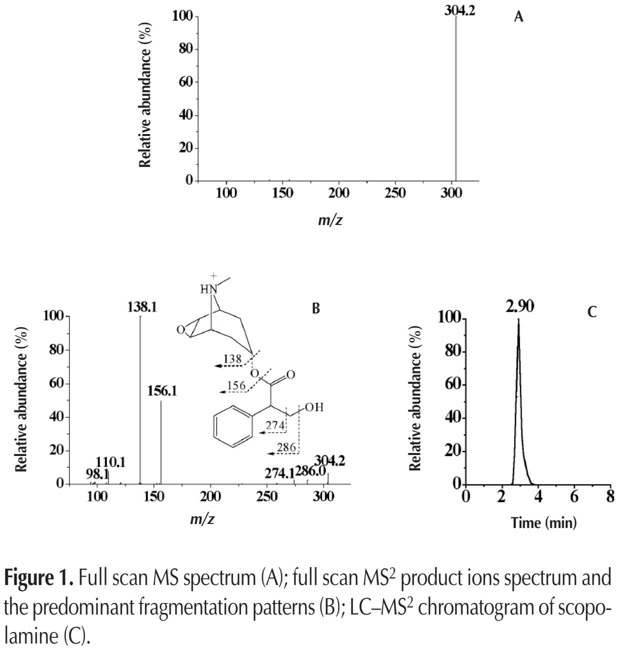

**Figure 1.** Full scan MS spectrum (A); full scan MS$^2$ product ions spectrum and the predominant fragmentation patterns (B); LC–MS$^2$ chromatogram of scopolamine (C).

*Preparation and incubation of homogenated liver*

Wistar rats (200 g) were fasted for 24 h and killed by decapitation between 10 a.m. and noon. A weighed amount of liver was rapidly placed on ice. It was rinsed twice with saline, immediately minced with scissors, and homogenated in ice-cold Krebs-Henseleit buffer (pH 7.4) (29) after sterilization to yield liver homogenate (0.4 g/mL). All the previously mentioned steps were carried out at 0~4°C. The concentration of P450 was detected by spectrophotometer (30). Scopolamine was added to liver homogenate to the concentration of 50 µg/mL. The mixture was incubated at 37°C with shaking. The incubation time was varied from 0, 30, 60, 90, 120, to 240 min. The gas phase was oxygen in all experiments. The incubation was terminated and extracted (twice) with equal volume of ethyl acetate. The organic extracts were combined and evaporated at 37°C under a slow stream of nitrogen. The residues were reconstituted in 0.6 mL of mobile phase and centrifuged at 13000 × *g* for 10 min. The supernatant was used for LC–MS$^n$ analyses. The blank experiment was carried out under the same conditions by replacing the liver homogenate with Krebs-Henseleit buffer.

### HPLC conditions

A reversed-phase column (Zorbax Extend-C18, 3.0 × 100 mm i.d., 3.5 µm, Agilent) was connected with a guard column (cartridge 2.1 × 12.5 mm, 5 µm, Agilent) filled with the same packing material to separate scopolamine and its metabolites in rat feces, urine, and plasma. The temperature of the column was set at 40ºC. The mobile phase consisted of methanol and 2mM ammonium acetate (adjusted to pH 3.5 with formic acid) (70:30, v/v). The flow rate was 0.2 mL/min during the whole run.

### MS conditions

MS detection was carried out in positive ion mode, and only the analyses of tropic acid were carried out in negative ion detection mode. Nitrogen was used as the sheath gas (40 arbitrary units). The MS analyses were performed under automatic gain control conditions, using a typical source spray voltage of 4.5 kV, a capillary voltage of 21 V, and a heated capillary temperature of 175°C. The other parameters, including the voltages of octapole offset and tube lens offset, were also optimized for maximum abundance of the ions of interest by the automatic tune procedure of the instrument. The MS$^n$ product ion spectra were produced by collision induced dissociation of the protonated molecular ion $[M+H]^+$ or the deprotonated molecular ion $[M–H]^–$ of all analytes at their respective HPLC retention times. Data acquisition was performed in full scan LC–MS and tandem MS modes.

## Results and Discussion

The in vivo and in vitro metabolism pathway of scopolamine was investigated. Blank samples and substrate were analyzed for the identification of the metabolites in biological samples.

### LC–MS and LC–MS$^n$ analyses of substrate

Full scan mass spectral analysis of scopolamine showed a protonated molecular ion of *m/z* 304 (Figure 1A). The MS–MS product ion spectrum of the protonated molecular ion (*m/z* 304) and its proposed fragmentation pathway are shown in Figure 1B. Scopolamine was eluted at 2.90 min under the experimental conditions (Figure 1C). Fragmentation of the protonated molecular ion of scopolamine in the ion trap led to five main product ions *m/z*: 286, 274, 156, 138, and 110. The product ions at *m/z* 286 and 274 were formed by the loss of $H_2O$ and HCHO from the molecular ion at *m/z* 304, respectively. The most abundant product ion at *m/z* 138 was formed by the loss of tropic acid ($C_9H_{10}O_3$, 166 Da). The ion at *m/z* 156 was produced by the loss of $C_9H_8O_2$ (148 Da). The fragment ions at *m/z* 156, 138, and 110 coexisted in the MS$^3$ spectra of *m/z* 286 and 274. It could be concluded that the ions at *m/z* 156 and 138 were a pair of characteristic product ions of scopolamine, and 148 Da and 166 Da were its characteristic neutral losses. These characteristic product ions and neutral losses were the sound bases to identify metabolites of scopolamine.

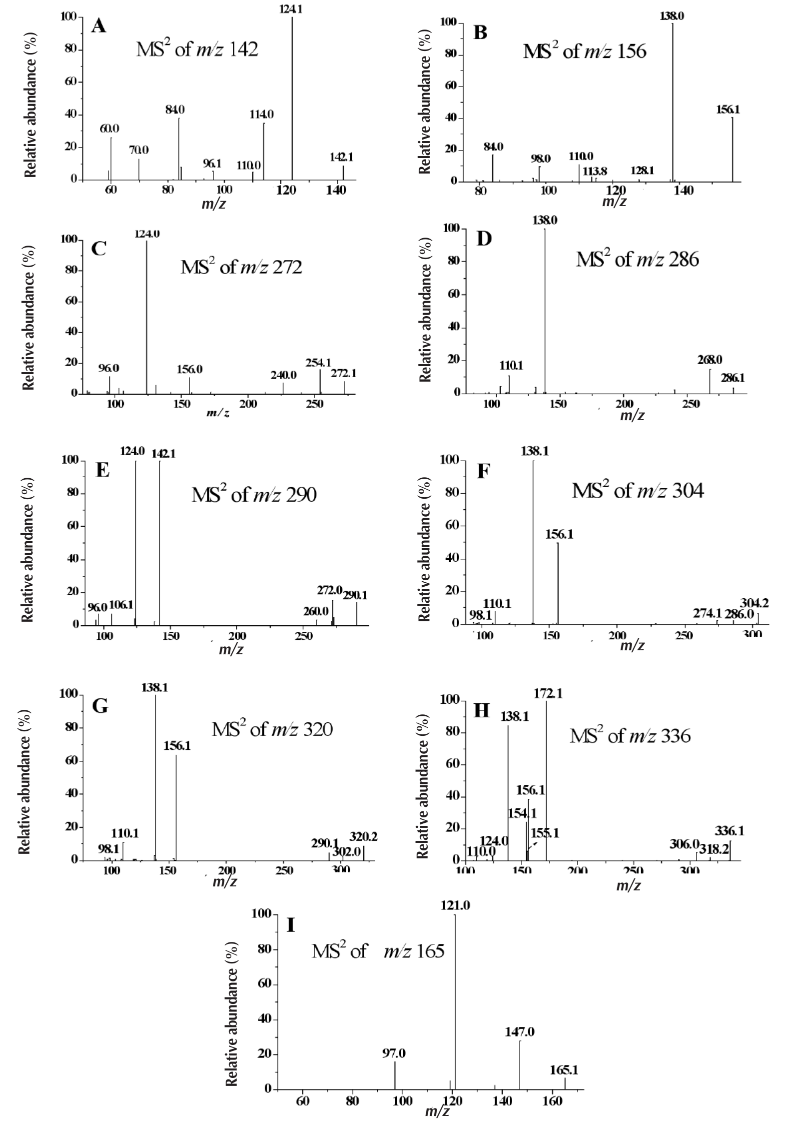

**Figure 2.** MS–MS product ion spectra of scopolamine and its 8 metabolites in rat feces.

### In vivo metabolism

*Identification of metabolites in rat feces*

Based on the method previously described, the parent drug and eight metabolites were found in rat feces after administration of scopolamine. Their protonated molecular ions ($[M+H]^+$) were at *m/z* 142, 156, 272, 286, 290, 304, 320, and 336, respectively. The MS–MS spectra of these analytes were presented in Figure 2. Among them, the retention time (Table I) and the MS and MS$^2$ spectra of the protonated molecular ion at *m/z* 304 (M0, Figure 2F) were the same as those of scopolamine. Therefore, M0 could be confirmed as the unchanged parent drug.

The MS$^2$ spectrum of *m/z* 156 (M2) was the same as the MS$^3$ spectrum of the protonated molecular ion of scopolamine at *m/z* 304 → 156, and there were the characteristic product ions at *m/z* 98, 110, 138 in its MS$^2$ spectrum (Figure 2B). So, M2 was identified as the hydrolysis product of scopolamine, and it was scopine.

The protonated molecular ion at *m/z* 142 (M1) and its daughter ions at *m/z* 124, 114, 96, 84, and 70 (Figure 2A) were all 14 Da less than *m/z* 156 (M1) and its daughter ions at *m/z* 138, 128, 110, 98, and 84, respectively. These results indicated that M1 should be the *N*-demethyl product of M2 (norscopine).

The characteristic product ions of *m/z* 110 and 138 appeared in the MS$^2$ spectrum of the protonated molecular ion at *m/z* 286 (M4, Figure 2D), which was decreased by 18 Da compared to that of the unchanged scopolamine. The result indicated that M4 should be the dehydrated metabolite of scopolamine (aposcopolamine). The *m/z* 268 ion may be produced by the loss of $H_2O$ from *m/z* 286 via enolization.

The protonated molecular ion at *m/z* 272 (M3) and its daughter ions at *m/z* 254, 124, and 96 (Figure 2C) were all 14 Da less than *m/z* 286 and its daughter ions *m/z* 268, 138 and 110, respectively. Therefore, M3 could be identified as the *N*-demethyl product of M4 (aponorscopolamine). The *m/z* 254 ion may be produced by the loss of $H_2O$ from *m/z* 272 via enolization.

The fragment ions at *m/z* 142 and 124 were produced by losing neutral fragments 148 Da and 166 Da from the parent ion at *m/z* 290 (M5, Figure 2E), which were the same as the neutral losses of the parent drug. It was obvious that the *m/z* 290 ion and its daughter ions at *m/z* 272, 260, 142, 124, and 96 were all 14 Da less than the molecular ion of parent drug (*m/z* 304) and its daughter ions at *m/z* 286, 274, 156, 138, and 110. Thus, M5 could be identified as the *N*-desmethyl product of scopolamine (norscopolamine).

The protonated molecular ion at *m/z* 320 (M6) was increased by 16 Da compared to that of the unchanged scopolamine. Because of the appearances of the characteristic fragment ions at *m/z* 156, 138, and characteristic neutral losses 164 Da (148+16) (*m/z* 320 → 156), 182 Da (166+16) (*m/z*

320 → 138) in its MS$^2$ spectrum (Figure 2G), M6 should be the hydroxylation product of scopolamine hydroxylated at the tropic acid part. The *m/z* 302 ion was produced by the loss of $H_2O$ from the parent ion at *m/z* 320. The results indicated that the benzyl hydrogen still existed in M6. Therefore, M6 was phenolic metabolites.

The characteristic product ions at *m/z* 156, 138, and 110 appeared in the MS$^2$ spectrum of the molecular ion at *m/z* 336 (M7, Figure 2H) that was increased by 32 Da compared to that of the parent drug. The appearance of the predominant product ion at *m/z* 172 (156+16) in the MS$^2$ spectrum of the molecular ion at *m/z* 336 indicated that the scopine part was oxidized, and the other oxidation should occur at tropic acid part. Besides, in the MS$^2$ spectrum of *m/z* 336, a pair of product ions at *m/z* 155 and 154 (more abundant than *m/z* 155) were produced by the loss of 17 and 18 Da from the ion at *m/z* 172, respectively. P.H. Cong (31) theoretically expounded the fragmentation feature of *N*-oxide: losing 17, 18 Da from the parent molecule and the fragmentation feature was validated using oxymatrine in our experiment. Based on these data, M7 was deduced as the *N*-oxide of scopolamine. The *m/z* 318 ion was produced by the loss of $H_2O$ from its parent ion at *m/z* 336. The results indicated that the benzyl hydrogen still existed in M7. So, M7 should be the hydroxyscopolamine *N*-oxide.

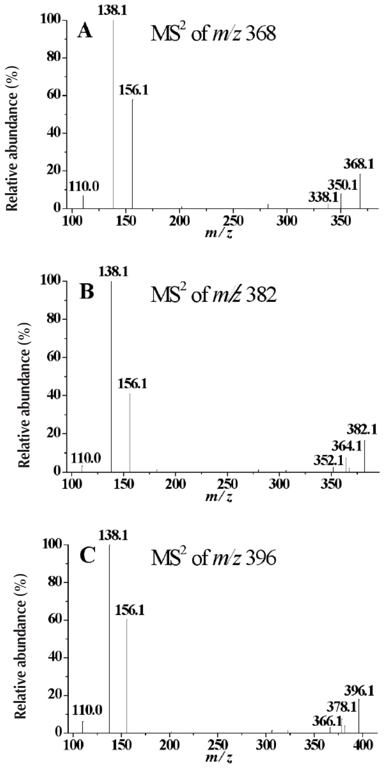

**Figure 3.** MS–MS product ion spectra of 3 new metabolites in rat urine.

The *m/z* 165 ion (M8) appeared in the negative ion full scan LC–MS spectrum of the urine samples. The appearances of the product ions at *m/z* 147 ($[M–H–H_2O]^-$) and 121 ($[M–H–CO_2]^-$) indicated that M8 was the hydrolysis product of scopolamine (tropic acid), which was in accordance with to the result of Wada et al. (25,26). No sulfate or glucuronide conjugate of M8 was found in rat feces.

Various solvents were used for the liquid–liquid extraction of scopolamine and its metabolites in rat feces. The analytical results were almost the same when ethyl acetate was substituted by chloroform or carbon dichloride. However, the ethyl acetate layers were supernatant and easy to decant, so ethyl acetate was used for the liquid–liquid extraction of scopolamine and its metabolites in rat feces.

### *Identification of metabolites in rat urine*

We found 18 metabolites (norscopine, scopine, tropic acid, aponorscopolamine, aposcopolamine, norscopolamine, hydroxyscopolamine, hydroxyscopolamine *N*-oxide, *p*-hydroxy-*m*-methoxyscopolamine, trihydroxyscopolamine, dihydroxy-methoxyscopolamine, hydroxyl-dimethoxyscopolamine, glucuronide conjugates, and sulfate conjugates of norscopolamine, hydroxyscopolamine, and the parent drug) in rat urine after administering scopolamine to healthy rats. In our study, three new metabolites were found in rat urine for the first time. Their MS–MS spectra are presented in Figure 3.

The protonated molecular ion at *m/z* 368, 382, and 396 was increased by 64 (16 × 4) Da, 78 (16 × 3 + 30) Da, and 92 (6 × 2 + 30 × 2) Da compared to that of unchanged scopolamine. The characteristic fragment ions at *m/z* 156, 138, and 110 appeared in their MS$^2$ spectra (Figure 3), and there were not the *m/z* 172 (156+16) ion in their MS$^2$ spectra. This showed that the tropine structure was retained in these metabolites. The appearance of their dehydrated fragment ions ($[M+H–H_2O]^+$) at 350, 364, and 378 in the MS2 spectra showed that they were phenolic metabolites. Therefore, the 3 metabolites could be identified as tetrahydroxyscopolamine, trihydroxy-methoxyscopolamine, and dihydroxy-dimethoxyscopolamine.

**Table I. The Comparative Distribution of Metabolites Identified From Different Matrices***

| Analyte | $[M+H]^+$ | Rt (min) | Feces | Urine | Plasma | Intestinal flora | Homogenized liver |
|---|---|---|---|---|---|---|---|
| M1 | 142 | 2.25 | + | + | + | – | – |
| M2 | 156 | 2.35 | + | + | + | + | – |
| M3 | 272 | 3.15 | + | + | + | – | – |
| M4 | 286 | 3.05 | + | + | + | – | + |
| M5 | 290 | 2.39 | + | + | + | – | + |
| M0 | 304 | 2.91 | + | + | + | + | + |
| M6 | 320 | 2.32 | + | + | + | – | – |
| M7 | 336 | 2.30 | + | + | – | – | – |
| M8 | 165 $[M-H]^-$ | 2.11 | + | + | + | – | – |
| M9 | 368 | 2.63 | – | + | – | – | – |
| M10 | 382 | 2.35 | – | + | – | – | – |
| M11 | 396 | 2.40 | – | + | – | – | – |

* + indicates "found" and – indicates "not found".

*Identification of metabolites in rat plasma*

According to the previously mentioned method, the parent drug and its 7 metabolites were identified in rat plasma after administration of scopolamine. They were norscopine, scopine, tropic acid, aponorscopolamine, aposcopolamine, norscopolamine, and hydroxyscopolamine.

The LC–MS$^2$ analysis of plasma samples showed that the parent drug and its 7 metabolites existed in plasma between 0.75 and 18 h after administration, and only scopine, aposcopolamine, norscopolamine, and tropic acid were found in plasma at 0.24 h. Norscopine, scopine, hydroxyscopolamine and tropic acid were found in plasma at 25 h.

**In vitro metabolism**

The substrate was incubated with intestinal fractions and liver homogenate of rats. Substrate was observed, but only one metabolite (scopine) was detected in the rat intestinal part. Another hydrolysis product of scopolamine (tropic acid) was not found in the incubation. The results showed that the metabolism of scopolamine was very weak in rat intestinal bacteria. Two metabolites (aposcopolamine and norscopolamine) were found in the liver homogenate incubation.

On the basis of this discussion and combining the results with those we have reported elsewhere (27), the metabolic pathway proposed for scopolamine in rats is depicted in Figure 4. The comparative distribution of metabolites identified from different matrices is shown in Table I.

In this work, the sensitivity of the method was determined using scopolamine. The limit of detection (LOD) was approximately 5 ng/mL by LC–MS$^n$. The specificity of the assay was evaluated by analyzing blank solution and blank feces, urine, and plasma samples of rats. No impurities or endogenous interferences were found. Therefore, the proposed method is highly sensitive and special for the quantitative determination of scopolamine and its metabolites.

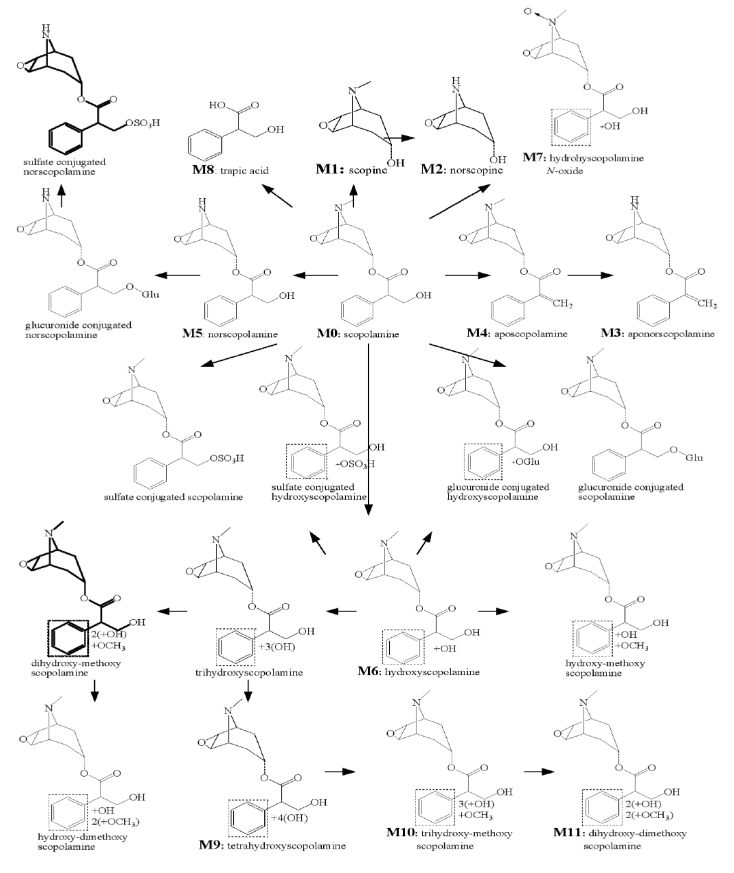

**Figure 4.** Proposed metabolic pathway of scopolamine in rat (Glu = flucuronic acid).

## Conclusion

In the present study, in vivo and in vitro metabolism of scopolamine was studied extensively. Metabolites were resolved, identified, and characterized using a highly specific and selective technique of LC–MS$^n$ ion trap with electrospray ionization in the positive ion mode. For the first time, 3 new metabolites were found in rat urine after ingestion of scopolamine, and 8, 7, 1, and 2 metabolites were identified in rat feces, plasma, intestinal bacteria, and liver homogenate. Various extraction solvents were compared for investigation into the metabolism of scopolamine.

## Acknowledgments

This work was supported by the Ministry-of-Education Key Laboratory for the Synthesis and Application of Organic Functional Molecules Natural Science Foundation of Hubei University (No.020-044109) and the Specialist Fund of Hubei University (020091130-ky2006004). The authors would like to thank their colleagues for their valuable technical assistance.

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Manuscript received October 31, 2006;
Revision received March 8, 2007.