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Determination of Mequitazine in Human Plasma by Gas-Chromatography/Mass Spectrometry with Ion-Trap Detector and Its Pharmacokinetics after Oral Administration to Volunteers

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The objective of this study was to develop an assay for mequitazine (MQZ) for the study of the bioavailability of the drug in human subjects. Using one mL of human plasma, the pH of the sample was adjusted and MQZ in the aqueous phase extracted with hexane; the organic layer was then evaporated to dryness, reconstituted and an aliquot introduced to a gas chromatograph/mass spectrometer (GC/MS) system with ion-trap detector. Inter- and intra-day precision of the assay were less than 15.1 and 17.7%, respectively; Inter- and intra-day accuracy were less than 8.91 and 18.6 %, respectively. The limit of quantification for the current assay was set at 1 ng/mL. To determine whether the current assay is applicable in a pharmacokinetic study for MQZ in human, oral formulation containing 10 mg MQZ was administered to healthy male subjects and blood samples collected. The current assay was able to quantify MQZ levels in most of the samples. The maximum concentration (C_{max}) was 8.5 ng/mL, which was obtained at 10.1 h, with mean half-life of approximately 45.5 h. Under the current sampling protocol, the ratio of $AUC_{t\to last}$ to $AUC_{t\to\infty}$ was 93.4%, indicating that the blood collection time of 216 h is reasonable for MQZ. Therefore, these observations indicate that an assay for MQZ in human plasma is developed by using GC/MS with ion-trap detector and validated for the study of pharmacokinetics of single oral dose of 10 mg MQZ, and that the current study design for the bioavailability study is adequate for the drug.

Key words: Mequitazine, Pharmacokinetics, Gas-chromatography/mass spectrometry, Ion-trap detector, Bioavailability, GC/MS, MQZ

INTRODUCTION

Mequitazine (MQZ, 10-3(3-quinuclidinylmethyl)phenothiazine) is a new phenothiazine derivative which has been used as a potent H_1 antagonist with few or no sedative side-effects (Uzan *et al.*, 1979). The antihistamine drugs act *via* competitive antagonism of histamine at H_1-receptors. Thus, ability of most of these agents to antagonize endogenously released histamine depends on the local concentrations of histamine and those of antagonists. H_1 antagonists are most useful in acute exudative types of allergy that presents with symptoms of rhinitis, urticaria, and conjuntivitis. Other allergies of the respiratory tract are more amenable to therapy with H_1 antagonist. The best results are obtained in seasonal rhinitis and conjuntivitis, in which this drug relieves the sneezing, rhinorrhea, and itching of eyes, nose, and throat (Wihl *et al.*, 1985).

MQZ analysis in dosage forms was made by using HPLC/UV and spectrophotometer, usually with sensitivity of higher concentrations than 1 μg/mL MQZ (El-Ragehy *et al.*, 2002). Most of the analytical method of MQZ in serum and urine was conducted by using a quadrupole mass analyzer type of gas-chromatography/mass spectrometry (GC/MS) after repeated administration of MQZ (Fourtillan *et al.*, 1984) or a single dose administration (Ylitalo *et al.*, 1989) since high sensitivity is required for the determination of MQZ concentrations.

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The selectivity and specificity of GC and HPLC methods are not sufficient for unequivocal identification of a compound. In the case, the GC/MS method is a good choice as it improves sensitivity and selectivity over existing analytical methods as well as identification of target compounds. Although various applications of the ion-trap detector to forensics and drug-abuse testing also have been reported (Pocci *et al.*, 1992; Wu *et al.*, 1992), no reports about MQZ are found.

Few pharmacokinetic data of MQZ in human is found due to difficulty in the determination of plasma MQZ concentrations, and better understanding of the MQZ pharmacokinetic behavior is necessary. This work is described firstly by using a GC/MS with an ion-trap detector in human plasma of volunteers orally administered 10 mg MQZ for its pharmacokinetic analysis.

MATERIALS AND METHODS

Chemicals and reagents

MQZ authentic standard and tablets (Primalan™, 5 mg) were obtained from Bukwang Pharmaceutical Co. (Seoul, Korea). Promethazine was purchased from Sigma (St.Louis, MO, U.S.A.). Analytical grade of hexane and methanol was purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). The other agents used for MQZ analysis were of analytical grade.

GC/MS instruments

MQZ concentrations in plasma were determined by using a GC/MS (Trace GC/Polaris Q, Thermo Finnigan, Austin, TX, U.S.A.) equipped with an ion-trap detector. An autosampler (AI 3000) was loaded on it. The instrument and data handling were operated by the support of Chemstation X-Calibur (Thermo Finnigan, Austin, TX, U.S.A.). Ultra-1 capillary column (17 m length × 0.2 mm inner diameter × 0.33 μm film thickness; Agilent Technologies, Palo Alto, CA, U.S.A.) was used. Oven temperature was set initially on 120°C, increased by 30°C per min to 240°C for 1 min, and finally increased by 15°C per min to 300°C, where staying for 3 min. Inlet and transfer line temperatures were 310 and 300°C, respectively. Splitless mode was selected. Ion source temperature was 250°C. Helium (99.999%) was used as a carrier gas at the flow rate of 0.8 mL/min. The detector was applied in the electron impact (EI) mode, being equivalent to ionization energy of 70 eV.

Study subjects

Male volunteers who submitted the agreement to attend this project were medically examined and 8 healthy volunteers were selected by a medical doctor in Bestian Hospital (Seoul, Korea), based on clinical examination including seropathological (hemoglobin, hematocrit, WBC, platelet), serochemical (blood urea nitrogen, creatinine, total protein, albumin, SGOT, SGPT, total bilirubin, cholesterol, glucose fasting, alkaline phosphatase), and urological (specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC) data. The subjects were instructed not to take any medicine for at least 1 week prior to and during the study period. Informed consent was completed by the subjects after explanation of the nature and aims of the work. They were accommodated to the same lodging facility one day before the blood sampling. They were fasted overnight before administration of the tablet. The study protocol was approved by the Institutional Review Board of Korea Institute of Science and Technology and by Korea Food and Drug Administration (KFDA).

Oral administration of MQZ tablets to human volunteers

A 21-gauge scalp-vein set was established on the arm vein of the volunteers, and 8 mL blood for blank sample was collected. According to the prescription directed by a doctor, 2 tablets of MQZ (10 mg) were orally taken to the designated group at random design (8 volunteers) with 150 mL of drinking water. No food was allowed until 4 h after dose administration. Lunch and dinner were provided to volunteers according to a time schedule. Beverages and caffeine were not allowed during the study. Blood was taken into a heparin-treated Vacutainer tube (Becton Dikinson, Rutherford, NJ, U.S.A.) at 0, 1, 3, 5, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 216 h after the oral administration. The time interval of blood sampling between volunteers was 2 min to consider blood sampling time. The blood was centrifuged to obtain plasma at 4°C. The plasma was stored at -70°C until analyzed.

Preparation of the calibration curve of MQZ

To a 15 mL centrifuging tube, 1 mL of the thawed blank plasma was added. And the various concentrations of MQZ were spiked to make the final concentrations of 0, 1, 2.5, 5, 10, 25, and 50 ng/mL. Promethazine of 10 ng (0.5 μg/mL, 20 μL) was added to the tube as an internal standard. And one half mL of sodium carbonate (1 M, pH 13) was added. After the tube was mechanically mixed on a vortex-mixer (Maxi Mix II, Thermolyne Co., Dubuque, IA, U.S.A.), 5 mL of hexane was added. The tube was vigorously agitated on a shaker (SM-25, Edmund Buhler, Germany) for 20 min and the organic layer was separated at 900 g for 10 min by centrifugation (Triac, Clay Adams, Rutherford, NJ, U.S.A.) and by freezing at -30°C in a deep freezer (Ecoline RE112, Lauda, Germany). The organic layer was transferred to another tube and evaporated on an evaporator under vacuum condition. The residue was dissolved in 50 μL of methanol. The solution of 2 μL was

applied to the GC/MS system. The calibration curve was prepared from the area ratios of the ion chromatogram of MQZ (m/z 124) to promethazine (m/z 72), and inter- and intra-day precision and accuracy were obtained.

Method validation

Within (intra-) and between (inter-) days precision and accuracy (bias %) were calculated from repeated analysis (n=5) of MQZ added in blank plasma, respectively. The limit of detection is a parameter of limit tests and may be determined as the smallest quantity of analyte that is expected to produce a response that is significantly different from that of a blank. The limit of quantitation may be defined as the smallest quantity of analyte that can be determined with acceptable precision and accuracy. The limit of quantitation was determined by diluting successively the lowest point of calibration and by performing within and between precision and accuracy tests (less than 20%).

Preparation of plasma samples

One mL of the thawed plasma obtained from healthy human volunteers was added to the 15 mL centrifuging tubes, followed by addition of internal standard promethazine of 10 ng (0.5 μg/mL, 20 μL). The tube was treated as described above. Based on the calibration curve of MQZ, the plasma concentrations of MQZ were determined from peak area ratios of MQZ to promethazine.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined from the time-plasma concentrations of MQZ by non-compartmental analysis by using WinNonlin software (Scientific Consulting Inc., Cary, NC, U.S.A.). In non-compartmental analysis, the highest concentration (C_{max}) and the time to reach the highest concentration (T_{max}) were read directly from the time-plasma concentration curves of MQZ. The area under the curve of time-plasma concentrations of MQZ until the last sampling time ($AUC_{0\to\infty}$) was determined by the equation of $AUC_{0\to\infty} = AUC_{0\to last} + C_{last}/\beta$, where β is the slope of the terminal phase of the time-log plasma concentration curve and C_{last} is the concentration at the last sampling time (Lee *et al.*, 1998).

RESULTS

Specificity

The base peaks for MQZ and internal standard promethazine were m/z 124 and m/z 72, respectively, and these ions were selected for quantitation. The other ions selected were m/z 322 and m/z 284 for the confirmation of MQZ and promethazine, respectively (these ion chromatograms not shown). Typical ion chromatograms (m/z 72 for internal standard; m/z 124 for MQZ) were shown in Fig. 1. The retention time is 3.6 min for promethazine and 5.1 min for MQZ. No interfering peaks were found in these retention times.

Effects of pH and solvents

MQZ and promethazine were extracted with hexane at various pHs to determine optimal pH value. As a result, MQZ showed higher peak areas at basic pH ranges (9-13) than at acidic pH, and the highest extraction was observed at pH 13. However, effects of pH on the extraction of the internal standard promethazine were less significant, resulting in similar peak areas over basic pH ranges. The highest extraction of MQZ among various solvents was made using hexane/isopropyl alcohol (97:3,

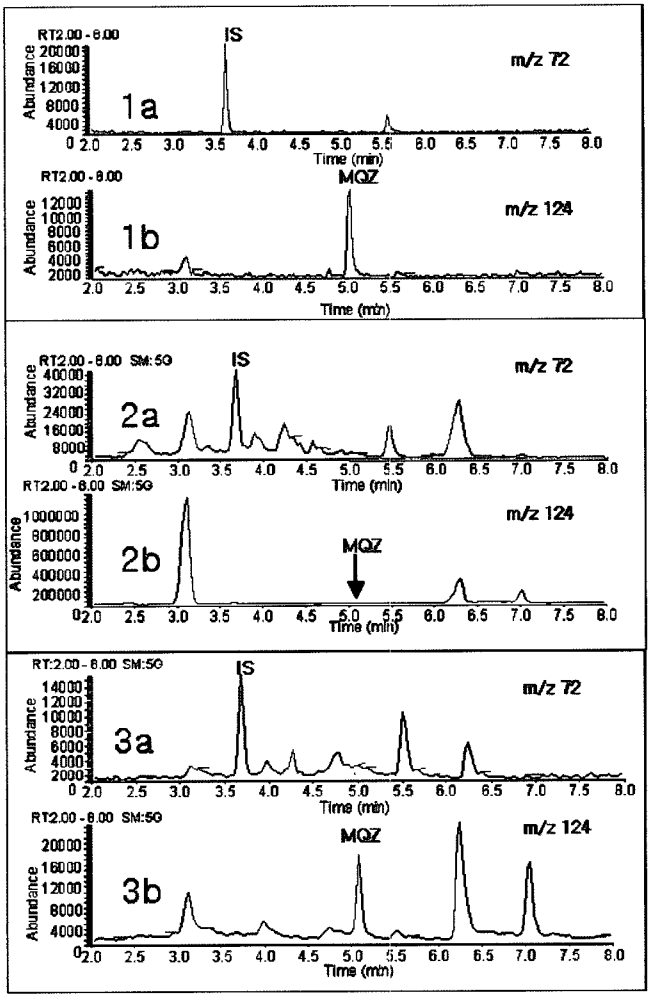

Fig. 1. The typical ion chromatograms of mequitazine (MQZ) and promethazine (IS) obtained by GC/MS with ion-trap detector from authentic standard (**1a** for mequitazine (m/z 72); **1b** for promethazine (m/z 124), plasma blank (**2a**, **2b**), and the plasma sample taken 5 h after oral administration of 10 mg mequitazine to a human volunteer (**3a**, **3b**). No interfering peaks were found at 3.6 min of promethazine (m/z 72) and 5.1 min of mequitazine (m/z 124).

v/v), and the second highest peak area was observed in hexane. However, MQZ was almost not extracted by ether or methylene chloride (Fig. 2).

Precision and accuracy

The validation data about precision and accuracy of MQZ were summarized in Table I. The detection limit of MQZ was decided to be 0.5 ng/mL, at which the signal to noise ratio was more than 3. The limit of quantitation was 1 ng/mL, at which it satisfies the analytical criteria that is defined as the lowest concentration yielding precision of less than 20% of coefficient variation and accuracy between 80 and 120% of the theoretical value (Table I). From 1 to 50 ng/mL MQZ, intra- and inter-day precisions were less than 17.69%, and the bias % for intra- and inter-day accuracies were less than 18.57%.

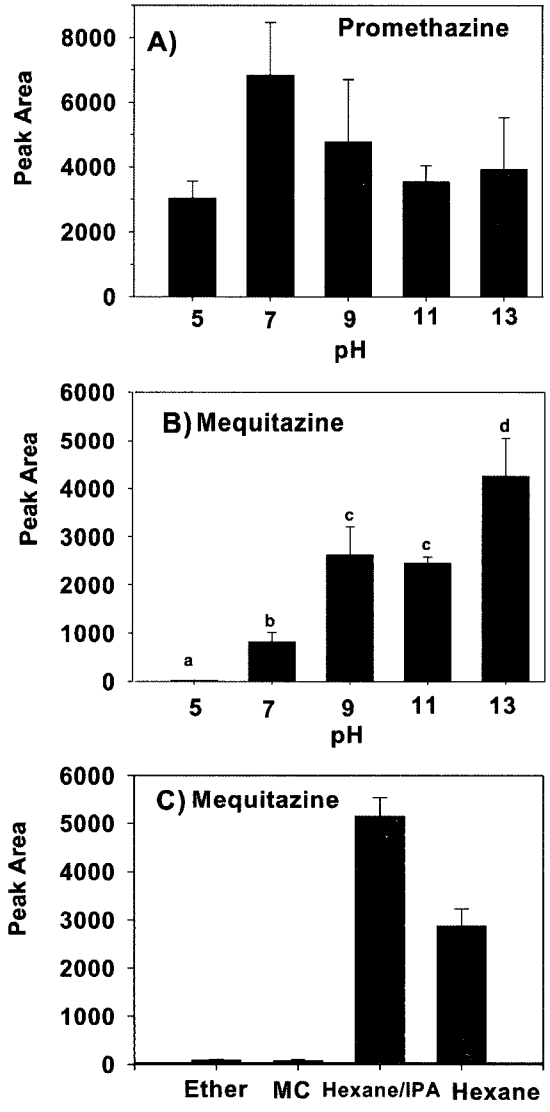

Fig. 2. Effects of various pH and solvents on the extraction of mequitazine. Promethazine was extracted with hexane at various pH ranges, showing that pH values are not significantly affected to the extraction of promethazine (**A**). Mequitazine is better extracted at basic pH ranges (**B**). Higher extraction was observed with hexane and hexane/isopropyl alcohol (97:3, v/v) mixture, compared to ether or methylene chloride (**C**). Abbreviations: MC, methylene chloride; IPA, isopropyl alcohol.

Table I. Intra-day and inter-day precision and accuracy for the determination of mequitazine in the plasma of human volunteers

Concentrations of mequitazine (ng/mL)	Precision (CV%)		Accuracy (bias%)	
	Intra-day	Inter-day	Intra-day	Inter-day
1	15.13	17.69	4.45	-18.57
2.5	12.19	12.34	2.73	2.30
5	8.22	10.71	8.91	10.22
10	10.72	10.01	-1.17	7.70
25	4.58	10.61	-0.19	-1.69
50	2.16	4.15	-2.18	-0.22

Mequitazine of 50 ng/mL was not included in the calibration curve shown in Fig. 3 because this point was beyond the range of plasma mequitazine concentration of volunteers.
Precision and accuracy data of intra- and inter-days were obtained from 5 repeated experiments.

Linearity

The linearity of MQZ calibration curve was determined by the linear least-square regression. The relative coefficient (r^2) was 0.9979 with the equation of y = 0.0483 x + 0.0300 at the range between 1 and 25 ng/mL of MQZ, showing a good linearity as shown in Fig. 3.

Pharmacokinetic analysis

Two MQZ tablets (primalan) were administered to 8 healthy male volunteers and blood sample were collected according to the scheduled time intervals. The mean time-plasma concentration curves of MQZ are shown in Fig. 4 and its pharmacokinetic parameters were calculated by non-compartmental analysis of the plasma concentration-

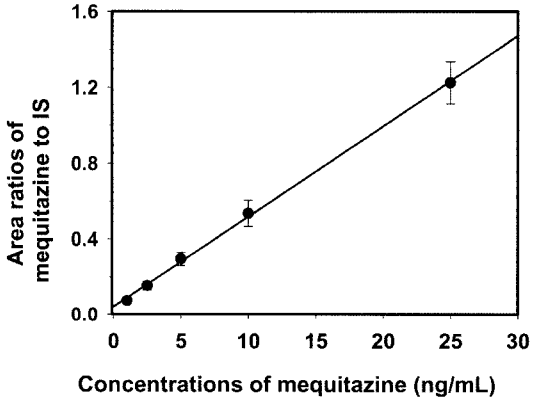

Fig. 3. Calibration curve of mequitazine

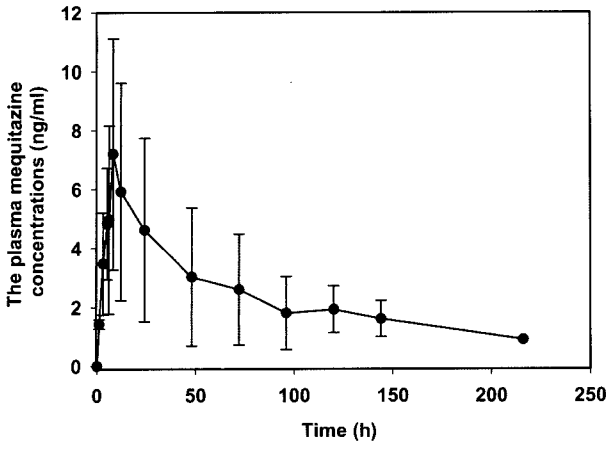

Fig. 4. Profiling of time-plasma concentrations of mequitazine after oral administration of a single dose (10 mg) mequitazine to 8 human volunteers. From these curves pharmacokinetic parameters of mequtazine were obtained as shown in Table II.

Table II. Pharmacokinetic parameters of mequitazine determined by non-compartmental analysis in 8 healthy male volunteers receiving 10 mg meqiutazine

Pharmacokinetic parameters	Mean ± SD (n=8)
$AUC_{0\rightarrow last}$, ng·h/mL	398.54 ± 243.15
$AUC_{0\rightarrow\infty}$, ng·h/mL	426.26 ± 278.11
C_{max}, ng/mL	8.48 ± 3.67
T_{max}, h	10.13 ± 6.20
K_e, h^{-1}	0.0163 ± 0.0044
$t_{1/2}$, h	45.53 ± 12.65

Pharmacokinetic parameters were described in the Materials and Methods section in detail.

time plot as shown in Table II.

In non-compartmental analysis, the ratio of $AUC_{t\rightarrow last}$ to $AUC_{t\rightarrow\infty}$ was 93.4%, indicating that blood sampling times were appropriately designed. The maximum concentration (C_{max}) was 8.5 ng/mL, which was obtained at 10.1 h (T_{max}) after the oral administration of 10 mg MQZ to volunteers. Its mean half-life was decided to be about 45.5 h.

DISCUSSION

MQZ is orally administered at low dosages, varying between 5 and 10 mg per tablet. The plasma levels are so very low at these dosages that a sensitive analytical method of MQZ in the plasma is required for pharmacokinetic analysis of MQZ. This work was conducted to develop the sensitive analytical method of MQZ in human plasma obtained from volunteers orally taken 10 mg MQZ.

The optimal condition for extracting MQZ in human plasma was achieved by using hexane or hexane/isopropyl alcohol (97:3, v/v) at basic pH ranges. We observed that MQZ was not extracted well with diethyl ether that had been used by Fortillan *et al.* (1984). This data indicate that MQZ is better soluble in hexane or hexane/isopropyl alcohol mixture than ether or methylene chloride, and at basic pH ranges.

In previous reports, MQZ was administered to human volunteers as multiple doses of 5 mg at 12 h intervals over 252 h. The obtained plasma and urine were determined by a GC/MS after extraction of the samples with 7 mL ether (Fourtillan *et al.*, 19984). However, this report did not describe pharmacokinetic parameters in detail except the mean half-life of 48.4 h. Ylitalo *et al.* (1989) had determined pharmacokinetic parameters over 72 h after oral administration of a single 5 mg dose of MQZ to healthy volunteers. The peak concentration of MQZ was 3.19 ng/mL at about 5.6 h and the elimination half-life was 45 ± 26 h.

In our work, the plasma concentrations were determined to 216 h post-dose, more than 4 times the half-life of MQZ. As a result, C_{max} was 8.48 ng/mL and T_{max} was 10.13 ng/mL. The mean half-life was decided to be 45.5 ± 12.7 h that was very similar to that previously reported in Ylitalo *et al.* (1989). A quadrupole mass analyzer type of mass selective detector has been used in most of the GC/MS instruments for the quantitation of drugs in biological samples. In this work, we determined the plasma concentration of MQZ by using ion-trap detector type of GC/MS that is less used, based on the comparison to the quadrupole mass analyzer type of GC/MS. This data is significant since MQZ was for the first time determined by using GC/MS with ion-trap detector and pharmacokinetic analysis was conducted over 216 h after oral administration of a single dose of 10 mg MQZ.

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