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PHARMACOKINETICS OF MANIDIPINE IN HEALTH VOLUNTEERS

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ABSTRACT

To establelish a HPLC-MS method for determining the concentrations of *Manidipine* in human plasma and to evaluate its pharmacokinetic characteristics. A Venusil XBP-C_8column column was used to separate *Manidipine* in plasma with a mobile phase of a mixture of 5mM ammonium acetate（0.5%acetic acid）- methanol - methy1 cyanide ((15：40：45, V/V/V) at a flow rate of 0.6ml.min^{-1}. Atmospheric pressure electronic spray ionization (AP-ESI) and ion mass spectral (m/z) of 611.4 were selected to quantify Manidipine, and 441.1 for *Nimodipine* (internal standard). The linear range of the standard curve of *Manidipine* was 0.25-50ng.ml^{-1}, and the determination limit was 0.25ng.ml^{-1}. The extraction recoveries were more than 49.85%, intra-day and inter-day RSD were less than 7.44%. The *Manidipine* plasma concentrations were determined after single and multiple dose and its pharmacokinetic parameters were calculated. The method is sensitive, fast and accurate. It is suiTablele for therapeutic *Manidipine* monitoring and its pharmacokinetic studies.

1. Introduction

Manidipine is calcium antagonist of dihydropyridine class and had no effects on the heart, will not affect the heart rate, has the function of atherosclerosis in some degree, has a protective effect of kidneys, does not affect the sympathetic nervous system, less adverse reaction, for hypertension merger in patients with type Ⅱ diabetes or glucose tolerance to reduce and elderly patients have good antihypertensive effect (Benchawan and Pornsak, 2016). *Manidipine* is unsTablele in plasma or exposed to light and the routine analytical method cannot meet the requirements of its pharmacokinetic studies in human body (Cheer and McclEllan, 2005; Naylere and Panagiotopoulos, 2001). A HPLC-MS method for determining the blood concentration of *Manidipine* with *Nimodipine* as internal standard was developed and report (Vitor et al., 2015). It is sensitivity, specialty and precision, and suiTablele for *Manidipine* therapy drug monitoring and pharmacokinetic studies (Fogari et al., 1999).

2. Materials and methods

2.1. Instruments and reagents

The HP1100LC-MS system was used to separate and detect *Manidipine* in human plasma, METTLER TOLEDO AX-205 electronic balance， XW-80A eddy mixer, PROINO high speed centrifuge, PK514BP ultrasonic cleaner were supplied by American Agilent Company, Mettler-Toledo Instrument (Shanghai) Co. Ltd, Shanghai Jingke Company,

American Kendro Laboratory Products and Germany Bandel Company, respectively.

Manidipine Hydrochloride Tablele of 10mg were offered by Jinan Limin Pharmaceutical Factory. Chinese Drug and Biological Products Quality Control institute provided the internal standards (IS) of *Manidipine* and *Nimodipine*. Methanol, methyl cyanide and ethyl acetate were all chromatographic pure grade.

2.2. Conditions for mass spectra and chromatogram

AP-ESI positive ion mode was used with atmospheric pressure of 50 psig and protective air of N_2 at a flow rate of 10L.min^{-1}, capillary voltage of 4000V，drying air temperature of 350℃. The selected iron monitoring (SIM) was used as ion collecting mode. The ion mass spectral (m/z) of 611.4 (M+1) was selected to quantify *Manidipine* and 441.1 (M+Na) for *Nimodipine.* Debris voltage 140 v and 120 v respectively. The separation was carried out with a mobile phase of a mixture of 5mM.l-1 ammonium acetate （0.5%acetic acid）-methanol- methyl cyanide ((15 : 40 : 45, V/V/V) at a flow rate of 0.6mL.min^{-1} and a sTablele phase of A Venusil XBP-C_8 column (150mm×4.6mm，5μm), 50μL of purified sample was injected (NguyenLan et al., 2016).

2.3. Method of pretreatment

The stock solutions of *Manidipine* and *Nimodipine* at the concentration of 0.1 mg.mL^{-1} and 0.12mg.mL^{-1} respectively were dissolved under methanol, kept from light during all course. A liquor of 1.0mL plasma of sample plus 50μL of IS was alkalinized by adding 0.1mL of 2 mol.mL^{-1} Sodium Hydroxide and 5mL of ethyl acetate。Then it was vortex-mixed 3 min, centrifuged at 4000 r·min^{-1} for 5 min .The water phase was discarded and 4mL of organic phase was moved to a clean glass tube and dried under Nitrogen in a 40 ℃ water bath. The residue was reconstituted with 0.1mL of mobile phase and 50μL of it was injected for analysis.

2.4. Corroboration of methods

Under above conditions, the retention time of IS and *Manidipine* were 6.7 and 4.5 min, respectively. The blank plasma, blank plasma spiked with IS plus *Manidipine*, volunteer samples spiked with IS, the SIM chromatograms and mass spectra of *Manidipine* were show in Fig 1 and Fig 2.

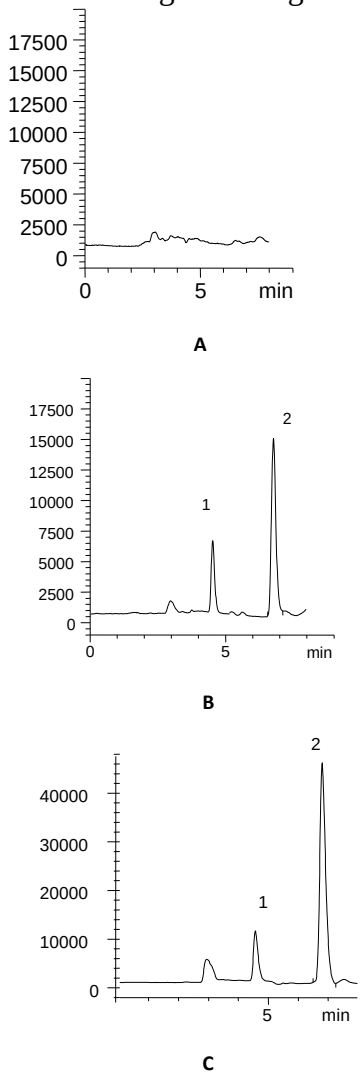

Figure 1. Chromatograms for the determination of *Manidipine* by HPLC

A: blank plasma；B: blank plasma plus *Manidipine* and internal standard; C: plasma sample of volunteer plus internal

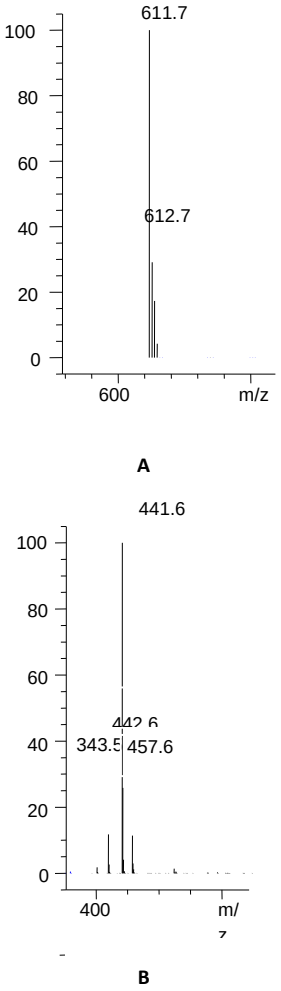

Figure 2. Mass spectra of control *Manidipine* and internal standard
A: *Manidipine*; B: internal standard

1.0 mL blank plasma was added in each glass tube which contained *Manidipine* at concentrations of 0, 0.25, 0.5, 1, 5, 10, 25 and 50 ng·mL^{-1} after dried with Nitrogen, purified, injected and analyzed. The regressive equation was as follows: Y=0.07481+20.29X, r= 0.99744, the limit of quantity (LOQ) is 0.25 ng·mL^{-1}.

0.5, 5 and 25 ng·mL^{-1} of *Manidipine* were spiked in blank plasma and analyzed at above conditions. The recovery rate, intra-day and inter-day RSD were calculated (Table1). After the samples which contained *Manidipine* at concentrations of 0.5, 5 and 25 ng·mL^{-1} were stored at -20℃ for 24 hours and 7 days, evaluated the affection of frost thawing and period of storage upon stableility of *Manidipine* (Table 2)

Table 1. Recovery rate, intra-day and inter-day relative standard deviation (RSD) of *Manidipine*(n=5)

Concentration /ng·ml-1	Recovery rate /%	Intra-day RSD /%	Inter-day RSD /%
0.5	49.92	4.15	3.08
5	49.85	3.21	7.76
25	50.50	7.44	2.41

Table 2. the data of sTableility test ($\bar{x}$ ±SD, n=5)

Concentration /ng.ml-1	Before frost thawing /ng.ml-1	frost thawing once /ng.ml-1	frost thawing twice /ng.ml-1
0.5	0.49±0.02	0.49±0.04	0.50±0.03
5	4.88±0.25	5.28±0.18	5.17±0.28
25	26.22±0.97	25.92±1.55	26.90±1.66

2.5. Subject and design

Twelve healthy volunteers were participated in this study after physical examination and laboratory screening. They were asked to avoid all prescription for at least 10 days before the study. Those who had a history of drug or alcohol abuse or allergy to the components of *Manidpine* Tablelets or capsules and those who had concomitant drug therapy were excluded. All subjects gave their written informed consent at the beginning of the study and being explained the nature of the drug and purpose of this study (Lifen et al., 2011).

A single dose of 10mg *Manidipine* normal Tablelet were given at am 7:00 after having a standard meal and 5mL blood samples were obtained before and 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0h after the administration of *Manidipine* normal Tablelet preparations. The blood samples were centrifuged and plasma were collected and stored at -20℃ for analysis (Lixin et al., 2014). After one-week washout period, a single dose of 20mg were given and experimentation was repeated.

The third week, each subject was orally taken 10mg *Manidipine* Tablelet at am 7:00 after having a standard meal for 7 days. Blood sample were collected from the 5th to 7th day before administration, and the 7th day after administration as same as those with single dose.

3. Results and discussions

3.1. Plasma concentrations of *Manidipine* in each group

The average plasma concentrations of manidipine after a sing and multiple doses administration were show in Table 3. The time-concentrations curves were show in Fig 3 and Fig 4.

Table 3. Mean plasma concentrations of *Manidipine* after a sing doses (10mg and 20mg) and multiple doses administration ($\bar{x}$ ±SD, n=12, ng·mL^{-1})

Time/h	Single dose of 10mg	Single dose of 20mg	multiple doses of 10mg
-48	-	-	0.358±0.137
-24	-	-	0.348±0.068
0	-	-	0.374±0.133
0.25	0.425±0.432	1.002±1.391	1.095±0.509
0.5	2.009±3.052	3.245±3.433	2.193±1.063
1.0	4.435±4.624	8.384±6.171	3.720±1.587
1.5	6.163±4.189	13.162±8.166	5.621±2.785
2.0	6.079±3.443	10.464±4.356	6.836±4.732
2.5	4.464±2.627	7.056±3.341	6.223±4.107
3. 0	3.185±1.706	5.276±2.394	4.430±2.702
4.0	2.232±0.988	3.407±1.552	3.301±1.839
6.0	1.547±0.508	2.220±0.794	2.153±0.820
8.0	0.965±0.378	1.430±0.437	1.339±0.469
12.0	0.554±0.146	0.722±0.153	0.805±0.259
24.0	0.311±0.068	0.368±0.087	0.335±0.095

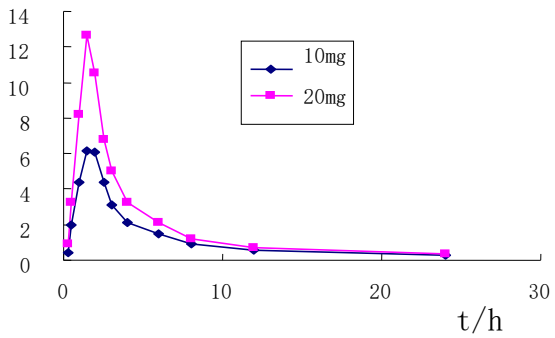

Figure 3. Mean time-concentration curves after a single dose of Manidipine

3.2. Pharmacokinetic parameters of manidipine in each group

The mean pharmacokinetic parameters of *Manidipine* in each group after a single and multiple doses administration were shown in Table 4 and Table 5.

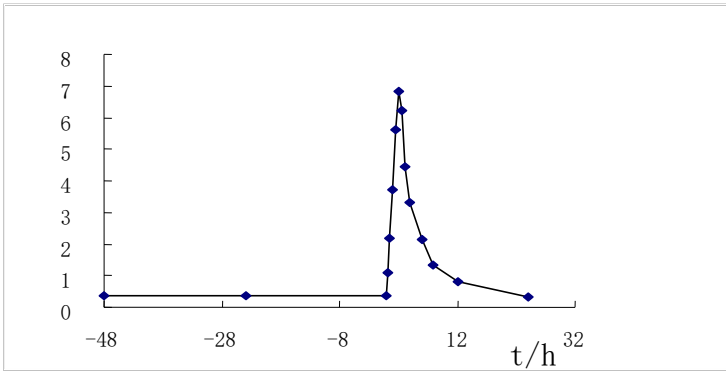

Figure 4. Mean time-concentration curves after multiple doses of Manidipine

Table 4. Pharmacokinetic parameters after a single dose of *Manidipine* ($\bar{x}$ ±SD, n=12)

parameters	10mg	20mg
$t_{1/2}$/h	6.67±2.83	6.89±2.66
C_{max}/ng·mL^{-1}	7.22±4.42	14.26±7.54
T_{max}/h	1.67±0.33	1.71±0.26
AUC_{0-24}/ng·mL^{-1}· h	29.42±12.92	46.69±18.19
$AUC_{0-\infty}$/ng·mL^{-1}·h	31.77±12.55	49.96±17.74

Table 5. Pharmacokinetic parameters after multiple doses of *Manidipine*($\bar{x}$±SD, n=12)

parameters	Coax/ng·mL^{-1}	Cmin/ng·mL^{-1}	Cav/ng·mL^{-1}
10mg	7.88±4.36	0.37±0.13	1.56±0.64
parameters	DF/%	AUCssng·h·mLl^{-1}	
10mg	4.60±0.88	37.39±15.34	

4. Conclusions

Manidipine is unsTablele in plasma or exposed in light. The blood concentrations of Manidipine is only 0.25-25 ng·mL^{-1}. The routine HPLC-UV method can't meet the requirement of pharmacokinetic studies and therapeutically monitoring of *Manidipine* in human body (NguyenLan et al., 2016). HPLC-MS has dual functions of both separation and detection and a strong anti-interference ability. The separation was carried out with a mobile phase of a mixture of 5mM·L^{-1} ammonium acetate（0.5%acetic acid）-methanol- methyl cyanide ((15 : 40 : 45, V/V/V) at a flow rate of 0.6ml·min-1 and a sTablele phase of A Venusil XBP-C_8 column (150mm×4.6mm, 5μm), 50μL of purified sample was injected. The ion mass spectral (m/z) of 611.4 (M+1) was selected to quantify *Manidipine* and 441.1 (M+Na) for *Nimodipine*. The period of analysis was only about 8 min. The determination of concentrations of *Manidipine* in human plasma by HPLC-MS method is sensitive and accurate (Tsukasa et al., 2006). In the process of experiment there was no interference of endogenous substances and there was no ion effect. The pharmacokinetic model of Manidipine belongs to two compartment model. So it can be used in the low limit detection for pharmacokinetic studies and therapeutically monitoring of *Manidipine* (Han and Fu Hong, 2006).

After taking *Manidipine* orally, the time to peak concentrations is about 1 to 2 hours, the half-life is about 6 hours, antihypertensive effect last 24 hours. It has a good peak/valley ratio of blood concentrations when taking *Manidipine* orally once a day (Francisco et al., 2011). When taking *Manidipine* orally after the meal, the peak concentration in plasma is 1.3 times and AUC is 1.6 times than those of an empty stomach, the time to peak concentration has no change (Saruta and Suzuki, 1999). Clearance in the body relate to the dose, AUC of taking 20 mg is 1.6 times than that of taking 10 mg (Zanchetti et al., 2001). *Manidipine* did not accumulate in plasma and the pharmacokinetic characteristics shows not significant difference between male and female volunteers.

Manidipine is new medicine chemicals with 3.1 classes. The test plan approved by the ethics committee and informed consent was signed before test.

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