Pharmacokinetic comparison of orally disintegrating, β-cyclodextrin inclusion complex and conventional tablets of nicardipine in rats

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Abstract: The goal of this study was to compare the pharmacokinetics of nicardipine hydrochloride orally disintegrating tablets and β-cyclodextrin inclusion complex with its conventional tablets. Forty-five rats were divided into three groups evaluating the effect of dosage forms on the pharmacokinetics of nicardipine hydrochloride. Blood samples were taken at predefined sampling points 0–24h after medication, and the plasma concentrations of nicardipine hydrochloride orally disintegrating tablets, β-cyclodextrin inclusion complex and its conventional tablets were determined by high-performance liquid chromatography. The orally disintegrating tablets increased in C_{max}, AUC and $t_{\beta 1/2}$ were observed, t_{max} occurred at 1.0 and 2.0h with orally disintegrating tablets and conventional tablets. The orally disintegrating tablets exhibited a longer elimination half-life ($t_{1/2\,\beta}$ 5.5h) compared with its conventional tablets ($t_{1/2\,\beta}$ 1.4 h). The mean dose corrected area under the plasma concentration-time curve extrapolated to infinity AUC (0−∞) of orally disintegrating tablets was 5.60 times greater than its conventional tablets. This research showed that formulation of nicardipine hydrochloride orally disintegrating tablets and β-cyclodextrins inclusion complex resulted in increase of bioavailability and prolong its activity time. [Life Science Journal 2010;7(2):80-84]. (ISSN: 1097-8135).

Keywords*:* Nicardipine hydrochloride; orally disintegrating; Pharmacokinetics; HPLC

Abbreviations：
C---concentration
t---time
NC--- Nicardipine hydrochloride
CDs ---Cyclodextrins
HPMC---hydroxypropyl methyl cellulose
L-HPC---low-substituted hydroxypropyl cellulose
CMC-Na---sodium carboxymethyl cellulose
PVPP --- poly (vinylpolypyrrolidone)
MCC--- microcrystalline cellulose
DSC---differential scanning calorimetry
FTIR --- fourier transformation-infraredHPLC
QC---quality control
HPLC---heigh perform liquid chromatography

1 Introduction

Nicardipine hydrochloride (NC), a calcium channel-blocking agent, is an effective drug in the management of mild to moderate hypertension, angina pectoris and cerebral disease (Pomponio et al. 2004; Catarina et al. 2002). However, the drug bioavailability is very limited (15 – 40 %) to short elimination half-life (about 1 h), and like other dihydropyridine derivatives, its standard formulation undergoes rapid absorption and extensive biotransformation in the liver, which often results in significant fluctuations in plasma concentrations (Catarina et al. 2002; Dollo et al. 1999).

To attain a prolonged therapeutic effect and a reduced incidence of side effects, sustained/controlled release formulations of NC have been developed to maintain a suitable plasma level for a long period of time, with minimal frequency of daily administration. NC microspheres using acrylic polymers (Zyazici et al. 1999), and NC microcapsules with ethylcellulose as a coating material have been prepared for this purpose (Catarina and Fernandesa 2003). Cyclodextrins (CDs), cyclic oligosaccharides with a hydrophobic central cavity that provides a microenvironment for appropriate sized non-polar molecules, are also strong candidates for achieving drug controlled release at the desired level. These carriers have been widely applied as multi-functional (Catarina et al. 2002). Pharmaceutical excipients due to their remarkable molecular complex property with many drugs modifying their physical, chemical and biological properties (Catarina et al. 2002; Uekama et al. 1998). The structure of nicardipine hydrochloride is showed in Figure 1. Then we used hydroxypropyl methyl cellulose (HPMC), low-substituted hydroxypropyl cellulose (L-HPC), sodium carboxymethyl cellulose (CMC-Na), poly (vinylpolypyrrolidone) (PVPP), microcrystalline cellulose (MCC) to prepare the orally disintegrating tablets not only to prolong its interaction time but improve stability and disintegrating time.

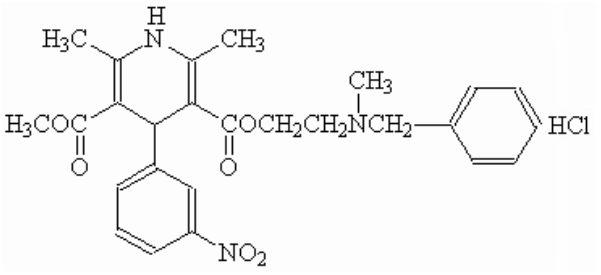

Figure 1. Chemical structure of nicardipine hydrochloride

2 Materials and Methods

2.1 Materials

Nicardipine hydrochloride (99.99%, purity) was a gift from Fusen Pharmaceutical Factory (Henan,China). Nicardipine hydrochloride orally disintegrating tablets, β-cyclodextrins inclusion complex and conventional tablets were prepared at our laboratory. HPMC, PVPP, L-HPC, CMC-Na, MCC were purchased from Shanghai Concord Tech Reagent Company (Shanghai, China). SD rats were purchased from the Center of Experimental Animals in Henan (Zhengzhou, China). Methanol of HPLC grade was purchased from Tianjin Concord Tech Reagent Company (Tianjin, China). All

the other reagents were of analytical grade.

2.2 Production of β-cyclodextrins inclusion complex

The preparation of NC-β-CD solidbinary systems was performed by different techniques (Kneading, Evaporation, Ultrasonic-Evaporation, Freeze-drying). We used differential scanning calorimetry (DSC) and fourier transformation-infrared (FTIR) spectroscopy to ensure the information of this chemical complex. In the end, the ultrasonic-evaporation (50℃, 3h, 100W) was chosen to produce the inclusion complex, the NC: β-CD (1:2) molar ratio based on the previous solubility studies (Sugimoto et al. 2001).

2.3 Preparation of nicardipine orally disintegrating tablets

Orthogonal test was used to confirm the optimum prescription, and according to the standard of Chinese Pharmacopoeia (2005) to product the orally disintegrating tablets and conventional tablets. The optimum prescriptions of single tablet is nicardipine hydrochloride β-cyclodextrins inclusion complex 60 mg, HPMC 7.5 mg, L-HPC 5.0 mg, MCC 30 mg, PVPP 12 mg, Magnesium stearate 0.15 mg, added to 35 mg mannitol .

2.4 Instrument conditions of HPLC

LC 2010A series (Shadows, Janpan) was used in the present work. Chromatographic separation was performed on a Diamonsil TM C_{18} column (250mm × 4.6mm I.D., 5 μm, Shadows, Janpan) at ambient temperature. The mobile phase consisting of a mixture of methanol and 0.01mol/L potassium dihydrogen phosphate buffer solution (90:10, v/v) was delivered at a flow rate of 1.0 ml/min. The injection volume was 20 μl and the detection wavelength was 237 nm.

2.5 Preparation of calibration standards and quality control samples

Stock solutions (1 mg/ml) of nicardipine hydrochloride and diethylstilbestrol (internal standard) were individually prepared in methanol. The stock solution of nicardipine hydrochloride was further diluted with methanol to give a series of standard solutions with concentration of 0.040, 0.18, 0.72, 2.5, 10, 40, 80, 320 μg/ml. Working solution of diethylstilbestrol was freshly prepared by diluting stock solution with methanol to give a concentration of 10 μg/ml.

Calibration standards of nicardipine hydrochloride were prepared by spiking appropriate amount of the standard solutions of nicardipine hydrochloride in blank plasma. Quality control (QC) samples were prepared at concentrations of 0.1, 40, 300μg/ml of nicardipine hydrochloride using the blank plasma.

2.6 Sample preparation

0.5 ml aliquot of each plasma sample was transferred to a 1.5 ml centrifuge tube, then centrifuged and added 10μg/ml of diethylstilbestrol. The supernatant was separated and 600μl of methanol were added, and shaken well, the contents were mixed by vortexing for 1 min and centrifuged for 10 min to separate the phases and evaporated under a stream of nitrogen at room temperature (Eiling et al. 2006). The residue was reconstituted with 200 μl of mobile phase and 20μl was injected into the HPLC column.

2.7 Method validation

Validation runs were conducted on six separate days. Each validation run consisted of a set of calibration standards at three concentrations over the concentration range and QC samples at three concentrations (0.1, 40, 300 μg/ml, n = 5 at each concentration) (Eiling 2006a; Dawes 2006b etc.). The results from QC samples were used to evaluate the accuracy and precision of the method developed. The analysis concentrations in plasma samples were determined by BAPP3.0 (supplied by China Pharmaceutical University)of the observed peak area ratios of the analysis and internal standard from the best-fit calibration curve. During routine analysis, each analytical run included a set of calibration standards, a set of QC samples in duplicate and plasma samples were determined.

The selectivity of the method was investigated by comparing chromatograms of blank plasma, standard plasma sample spiked with nicardipine hydrochloride (10μg /ml) and diethylstilbestrol (10μg/ml) and plasma sample after an oral dose of nicardipine hydrochloride orally disintegrating tablets and β-cyclodextrins inclusion complex and conventional tablets. An additional test was performed to demonstrate if there was any interference from the plasma matrix (Zhang et al. 2003). The test was performed as follows: the standard solutions of nicardipine hydrochloride at 2.5, 10, 40μg/ml were added in blank plasma after extraction and determined by the present HPLC method. Standard solutions of nicardipine hydrochloride at 0.1, 40, 300μg/ml were directly determined without extraction. Based on the percentage of peak area ratio of the analysis added in plasma after extraction was relative to that of the analyte without extraction, RE (%) was calculated to evaluate the accuracy of the determination without interferences from the matrix (Zhang et al. 2003).

The linearity of each calibration curve was determined by plotting the peak area ratio (y) of the analysis to internal standard versus the nominal concentration (x) of the analysis. The calibration curves were obtained by weighted ($1/x2$) linear regression analysis (Eiling et al. 2006).

The extraction recoveries of nicardipine were determined at 0.1, 40, 300μg/ml by comparing the responses from plasma samples spiked before extraction with the corresponding standard solutions without extraction.

2.8 Application of the method

The HPLC method was successfully applied in the pharmacokinetic studies of nicardipine hydrochloride orally disintegrating tablets, β-cyclodextrins inclusion complex and conventional tablets in 45 female SD rats of 200±20g in weight. The rats were randomly divided into three groups with fifteen rats which were divided into five of each group. The rats were fasted 12h before the test. 6, 12, 24mg/kg low-middle-high three doses of

nicardipine hydrochloride orally disintegrating tablets, β-cyclodextrins inclusion complex and conventional tablets were tested in this study. The rats in the three groups were given single dose of low-middle-high three doses with 9ml of warm water. Within 24 h after oral administration of the tablets, the rats had a standard diet while water intake was free . Blood samples (0.5 ml) were obtained immediately pre-dose and 5,10, 20, 30min, 1, 2, 4, 6, 8, 10, 12, 24h post-dose, which were collected in tubes previously treated with heparin and plasma was separated by centrifugation and kept frozen at −20℃ until analysis. Plasma concentrations of nicardipine were determined by the present HPLC method.

3 Results and Discussion

3.1 Method development

Liquid–liquid extraction was used for the sample preparation. This simple procedure produced a clean chromatogram for blank plasma sample and yielded satisfactory recoveries of the analytes from the plasma. In this work, 10% potassium dihydrogen phosphate buffer solution (0.01mol/l) were added in the process of sample preparation to adjust the medium to about pH 5.5 and thus to free the drug bases from their hydrochlorides for the following extraction by organic solvent. In this study, methanol and 0.01mol/L potassium dihydrogen ghosphate buffer solution (90:10, v/v) were used for extraction.

A Diamonsil TM C18 column (250mm × 4.6mm I.D., 5μm) was used. Other chromatographic conditions, especially the composition of mobile phase, were tested to achieve good resolution and symmetric peak shapes of analytes as well as short run time. Internal standard plays an important role in biopharmaceutical analysis and is often required to have similar physical and chemical properties with the analyte such as solubility and acid–base properties (p*K*a) (Eiling et al. 2006). On the basis of the above requirements, diethylstilbestrol was found to be suitable for the present work and finally used as the internal standard.

After liquid–liquid extraction, both analytes are present in drug base forms. For the analysis of basic drugs by HPLC methods, it was found that a mixture of methanol and 0.01mol/l potassium dihydrogen phosphate buffer solution (90:10, v/v) could achieve our purpose and was finally adopted as the mobile phase for the chromatographic separation. The retention times were 5.3 min for nicardipine and 3.0 min for diethylstilbestrol. The run time was less than 7min.

3.2 Selectivity

The results of selectivity demonstrated a clean chromatogram from blank plasma sample after sample preparation by liquid–liquid extraction (Figure 2) and the absence of endogenous interferences from the plasma matrix and the satisfying selectivity of the present method for the determination of nicardipine hydrochloride in rats plasma.

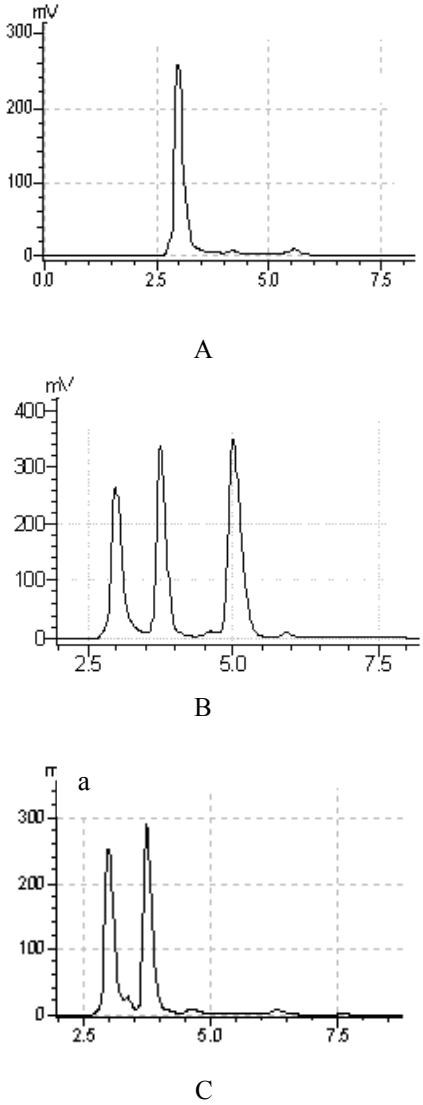

Figure 2. The chromatogram of separation: A. blank plasma, B. plasma sample added diethylstilbestrol (a-diethylstilbestrol; b-nicardipine hydrochloride), C. plasma sample

3.3 Linearity

To evaluate the linearity of the HPLC method, plasma calibration curves were determined in triplicate on six seperate days. Representative regression equation for the calibration curve was y = 0.7567x + 0.6555, r = 0.9998, for nicardipine hydrochloride. Good linearity was observed over the concentration range of 0.040-320 μg/ml.

3.4 Precision

The precision of the method were evaluated based on the data from QC plasma samples at three concentrations (0.1, 40, 300 μg/ml) in five validation runs. The intra-day and inter-day precision were expressed as the relative standard deviation (R.S.D.). As shown in Table 1, for each QC level of nicardipine hydrochloride, the intra-day and inter-day mean precisions (R.S.D.) were 0.45 and 7.69 %, indicating acceptable precision of the present HPLC method for the determination of nicardipine hydrochloride in rat plasma.

Table 1. The precision for the determination of nicardipine in rat plasma

Origin（μg/ml）	Intra-precision		Inter-precision	
	Found（μg/ml）	RSD（%）	Found（μg/ml）	RSD（%）
0.1	0.094	0. 78	0.11	5. 79
40	41.3	0.36	44.72	8.52
300	290	0.21	295	8.76

3.5 Extraction recovery and accuracy

The extration recovery of nicardipine hydrochloride from rat plasma was determined by comparing concentration from plasma samples spiked with the adding standard solutions concentration. The accuracy of nicardipine hydrochloride from rat plasma were determined by comparing peak areas from plasma samples spiked before extraction with the corresponding standard solutions without extraction. The results showed in table 2 at concentrations of 0.1, 40, 300 μg/ml of nicardipine hydrochloridel.

Table 2. Extraction recovery and accuracy for the determination of nicardipine in rat plasma

Origin (μg/ml)	Extraction-recovery		Accuracy		
	Found（μg/ml）	Recovery（%）	Normal- area	Sample-area	Accuracy（%）
0.1	0.094	94.0	6157	5497	89.3
40	41.3	103.3	2007621	1817562	90.5
300	298	99.3	18471000	16595000	89.8

3.6 Pharmacokinetics of nicardipine orally disintegrating tablets in rats

The present HPLC method achieved satisfactory results for the determination of nicardipine hydrochloride in rat plasma and was successfully applied in the pharmacokinetic study of nicardipine hydrochloride orally disintegrating Tablets following oral administration to rats. The result of the pharmacokinetics of orally disintegrating Tablets, conventional tablets, β-cyclodextrins inclusion complex shown in Table 3 and Figure 3.

Table 3. Pharmacokinetic parameters of orally disintegrating tablets, conventional tablet, β-cyclodextrins inclusion complex（mean±SD，n = 6）

Pharmacokinetic parameters	conventional tablet	β-cyclodextrins inclusion complex	orally disintegrating tablets
$AUC_{0-\infty}$ (μg h /ml)	123.15 ± 31.00	269.02 ± 67.00	689.10 ± 147.00
C_{max} (μg /ml)	41. 47 ± 13.11	44.38 ± 12.79	151.17 ± 31.06
T_{max} (h)	2 ± 0.12	1 ± 0.00	1 ± 0.00
$t_{½\beta}$ (h)	1.4 ± 0.01	4.1 ± 0.02	5.5 ± 0.04

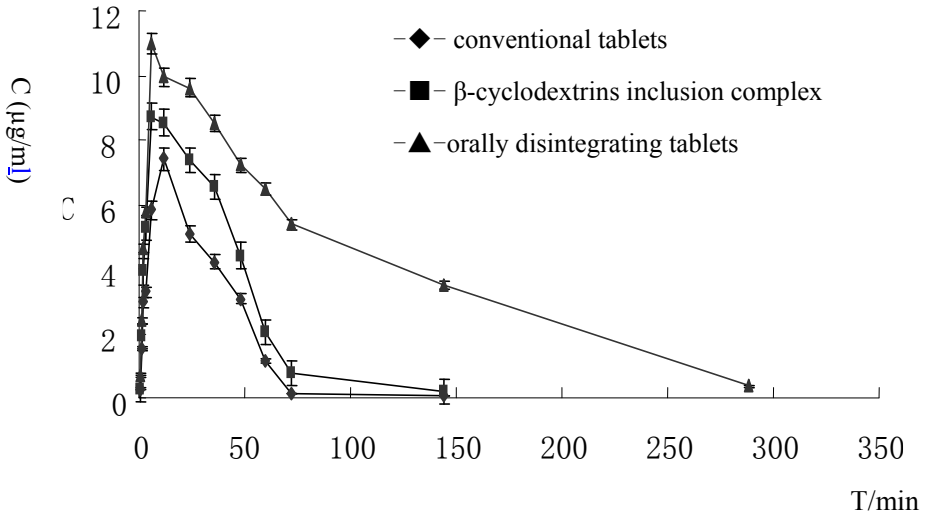

Figure 3. Concentration–time profiles of nicardipine hydrochloride orally disintegrating tablets, β-cyclodextrins inclusion complex and conventional tablets after oral administration of in rat plasma（mean±SD，n = 6）

The orally disintegrating tablets increased in C_{max}, AUC and $t_{1/2\beta}$were observed, t_{max} occurred at 1.0 and 2.0h with orally disintegrating tablets and conventional tablets. The orally disintegrating tablets exhibited a

longer elimination half-life ($t_{1/2\,\beta}$ 5.5h) compared with its conventional tablets

($t_{1/2\,\beta}$ 1.4h). The mean dose corrected area under the plasma concentration-time curve extrapolated to infinity AUC (0−∞) of orally disintegrating tablets was 5.60 times greater than its conventional tablets. The β-cyclodextrin inclusion complex were statistically significantly increased compared to the conventional tablets. This showed that formulation of nicardipine hydrochloride orally disintegrating tablets and β-cyclodextrins inclusion complex resulted in increase of bioavailability and prolong its activity time.

The pharmacokinetic results provide the proof of concept: nicardipine hydrochloride formulated as a medicated orally disintegrating tablets results in a statistically significant increase in its bioavailability as the results of this study. The main results from this study are orally disintegrating tablets and β-cyclodextrins inclusion complex formulation increased in the bioavailability compared to conventionall tablet, expressed as dose-corrected AUC (0−∞).

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References

1. Pomponio R, Gotti R, Fiori J. Photostability studies on nicardipine–cyclodextrin complexes by capillary electrophoresis. J Pharm.and Biomed Anal 2004; 35:267–275.
2. Catarina M, Fernandes J, Veiga B. Physicochemical characterization and in vitro dissolution behavior of nicardipine–cyclodextrins inclusion compounds. Eur J Pharm Sci 2002;15:79–88.
3. Dollo G, Corre P, Chollet M, Chevanne F. Improvement in solubility and dissolution rate of 1,2-dithiole-3-thiones upon complexation with β-cyclodextrin and its hydroxypropyl and sulfobutylether-7 derivatives. J Pharm Sci 1999; 88: 889–895.
4. Zyazici O, Sevgi F, Ertan G. Micrometric studies on dissolution characteristics of famotidine by inclusion in β-cyclodextrin-nicardipine hydrochloride microcapsules. Int J Pharm 1996; 138: 25–35.
5. Catarina M, Fernandesa. Hydrophilic and hydrophobic cyclodextrins in a new sustaine dreleaseoral formulation of nicardipine: invitro evaluation and bioavailability studies in rabbits. J Control Release 2003; 88:127–134.
6. Uekama K, Hirayama F, Irie T. Cyclodextrin drug carrier system.Chem Rec 1998; 98: 2045–2076.
7. Meiling Q, Peng W, Xin J. Liquid chromatography–mass spectrometry method for the determination of nicardipine in human plasma. J Chromatogr B 2006; 830: 81–85.
8. Dawes C. Absorption of urea through the oral mucosa and estimation of the percentage of secreted whole saliva inadvertently swallowed during saliva collection. Arch Orallly Biology 2006; 51:111–116.
9. Zhang YF, Chen XY, Zhong DF, Dong YM Pharmacokinetics of loratadine and its active metabolite descarboethoxy loratadine in healthy Chinese subjects. Acta Pharm Sci 2003; 24: 715–718.
1. 10. Sugimoto M, Matsubara K, Koida Y. The preparation of rapidly disintegrating tablets in the mouth Pharm Dev Technol 2001; 4: 487–493.

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