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# Determination of Concentration of Nifekalant in Human Plasma by HPLC Method and Investigation of Its Pharmacokinetics

Xin Xie[a], Changbin Li[b], Yali Wu*[c]

[a] Henan polytechnic college, Zhengzhou, Henan, China,
[b] Henan University of Animal Husbandry and Economy, Zhengzhou, Henan, China,
[c] Depart Guangdong Industry Technical College, Guangzhou, 510300, China.
ericascut@126.com

Objective: To establish a HPLC method for determining the concentrations of Nifekalant in human plasma and to evaluate its pharmacokinetic characteristics. Method: A ODS3 (250 mm × 4.6 mm 5 μm) column was used to separate Nifekalant in plasma with a mobile phase of a mixture of ammonium acetate (0.1 mol/L)-methanol-methyl cyanide (440:180:180, V/V) at a flow rate of 1ml/min. Nifekalant was detected at 270nm. The Nifekalant plasma concentrations were determined after intravenous injection and its pharmacokinetic parameters were calculated by DAS2.0. Results: The linear range of the standard curve of Nifekalant was 5~1000ng/mL, and the determination limit was 5 ng/mL. The extraction recoveries were more than 80%, intra-day and inter-day RSD were less than 6.23%. The main pharmacokinetics of Nifekalant after intravenous injections 0.3 mg/kg or 0.4 mg/kg Nifekalant Hydrochloride Injection were as follows, $t_{1/2}$ (1.545±0.382) h and (1.344±0.188) h, $T_{max}$ 0.08 h and 0.08 h, $C_{max}$ (230.946±54.023) ng/mL and (358.615±73.948) ng/mL, $AUC_{0\text{-}5}$ (193.526±45.194) ng/mL/h and (285.608±46.569) ng/mL/h, $AUC_{0\text{-}\infty}$ (209.895±48.117) ng/mL/h and (302.439±50.191) ng/mL/h, MRT (1.179±0.147) h and (1.128±0.085) h, CL(1.499±0.353) L/kg/h and (1.354±0.22) L/kg/h, V (3.402±1.443) L/kg and (2.596±0.353) L/kg, and after intravenous infusion of 0.4mg/kg/h Nifekalant Hydrochloride Injection were as follows, $t_{1/2}$ (1.348±0.227) h, $C_{max}^{ss}$ (444.303±88.122) ng/mL, $AUC_{0\sim12}$ (2609.020±498.200)ng/mL/h,$AUC_{0\sim\infty}$(2627.332 ±499.887) ng/ml/h, MRT (3.726±0.182) h, $T_{max}$ (1.603±2.259) h, CL (0.315±0.062 ) ng/mL/h, V (0.609±0.149) L/kg, respectively. Conclusions: The method is sensitive, fast and accurate. It is suitable for therapeutic Nifekalant monitoring and its pharmacokinetic studies.

## 1. Introduction

Nifekalant is a pure nonselective potassium channel blockers reported by Harayama et al. (2014), which can effectively control the Rapid ventricular arrhythmia caused by turn-back and has good curative effect on ischemic cardiac arrhythmia reported by MINAMI et al. (2004) and Shiga et al. (2001). Compared with other anti-arrhythmic drugs, Nifekalant is safer and more effective and the first selection medicaments of prevention and treatment ventricular arrhythmia for patients with organic heart disease which was confirmed (Ando et al. (2005)). A simple and selective high-performance liquid chromatography method was developed and validated for the determination of Nifekalant hydrochloride in canine plasma (Zhou et al. (2012) reported) .In this study HPLC method for determining the blood concentration of Nifekalant with Ornidazole as internal standard in chinese people plasma was developed and reported. No ion inhibition effect. It is sensitivity, specialty and precision, and suitable for Nifekalant therapy drug monitoring and pharmacokinetic studies.

## 2. Materials and methods

### 2.1 Instruments and reagents

The HPLC SIL-20A system with automatic sampler (LC-10AT) was used to separate and detect Nifekalant in human plasma, METTLER TOLEDO AX-205 electronic balance, XW-80A eddy mixer, PROINO high speed centrifuge, PK514BP ultrasonic cleaner were supplied by Japan SHIMADZU Company, Mettler-Toledo Instrument (Shanghai) Co. Ltd, Shanghai Jingke Company, American Kendro Laboratory Products and Germany Bandel Company, respectively.

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Nifekalant hydrochloride injection and reference substance were offered by Sichuan Baili Pharmaceutical Factory. Chinese Drug and Biological Products Quality Control institute provided the internal standards (IS) of Ornidazole. Methanol, methyl cyanide and ethyl acetate were all chromatographic pure grade.

**2.2 Conditions for chromatogram**

The separation was carried out with a mobile phase of a mixture of ammonium acetate (0.1 mol/L)-methanol-methyl cyanide (440:180:180, V/V) at a flow rate of 1ml/min and a stable phase of A INERTSIL ODS-3 column (250 mm × 4.6 mm 5 μm), 20 μl of purified sample was injected. Nifekalant was detected at 270 nm.The Conditions for chromatogram was reported by ZHANG et al. (2004) and CAO et al. (2004).

**2.3 Method of pretreatment**

The stock solutions of Nifekalant and Ornidazole at the concentration of 1 mg/mL were all dissolved under methanol and kept at 4 ℃, respectively. A liquor of 0.5 ml plasma of sample plus 40 μl of IS (Ornidazole 10 μg/mL) was acidified by adding 50 μl of 0.1 mol/mL hydrochloric acid and 4ml of ethyl acetate. Then it was vortex-mixed 2 min,centrifuged at 5000 r·min-1 for 5 min .The water phase was discarded and 4 ml of organic phase was moved to a clean glass tube and dried under Nitrogen in a 40 ℃ water bath. The residue was reconstituted with 0.1 mL of mobile phase, centrifuged for 3 min, and 20 μl of it was injected for analysis.

**2.4 Corroboration of methods**

Under above condition, the retention time of IS and Nifekalant were 4.689 and 9.764 min respectively. The blank plasm spiked with IS plus Nifekalant, volunteer samples spiked with IS were shown in Figure 1.

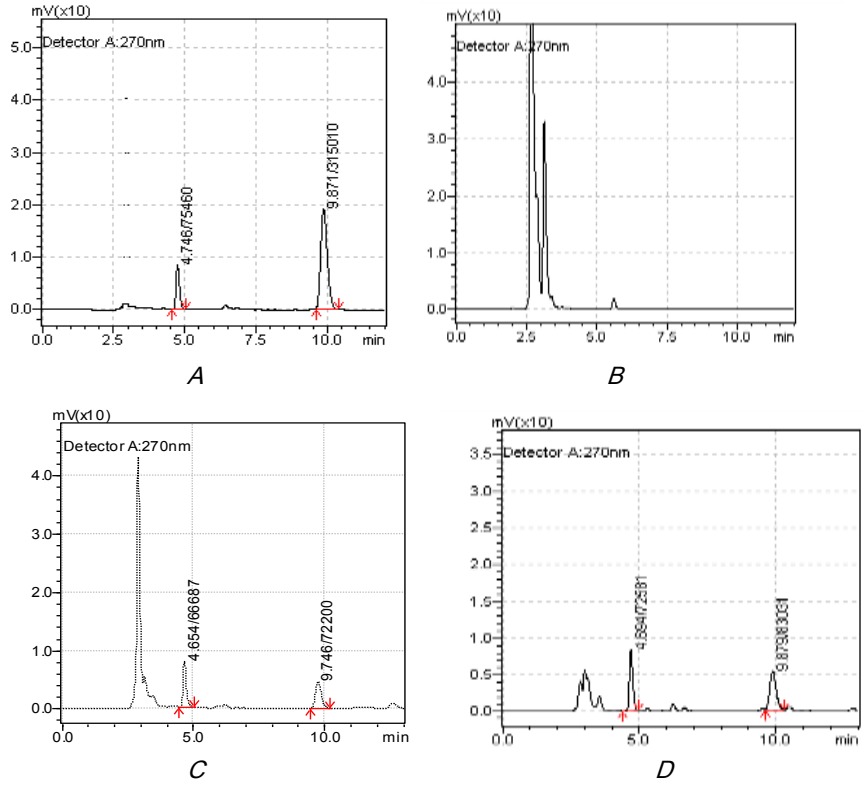


*Figure 1: The chromatograms of Nifekalant plus IS(A).blank plasma(B).blank plasma spiked with Nifekalant and IS(C). Volunteer plasma spiked with IS(D)*

0.5 mL blank plasma was added in each glass tube which contained Nifekalant at concentrations of 5, 10, 20, 50, 100, 200, 500 and 1000 ng/mL. After dried with Nitrogen, purified, injected and analyzed the regressive equation was as follows: Y= -0.01378+0.00473X, r = 0.9999, the limit of quantity (LOQ) is 5 ng/mL. The linear relationship of Nifekalant from 5ng/mL to 1000 ng/mL is good.

15, 200 and 750 ng/mL of Nifekalant were spiked in blank plasma and analyzed at above conditions. The recovery rate, intra-day and inter-day RSD were calculated (Table 1).

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

| Concentration (ng/mL) | Recovery rate (%) | Intra-day RSD (%) | Inter-day RSD (%) |
|---|---|---|---|
| 15 | 89.465 | 2.071 | 6.228 |
| 200 | 94.086 | 2.873 | 5.116 |
| 750 | 80.375 | 1.326 | 4.384 |

**2.5 Subject and design**

16 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 nifekalant 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.

16 healthy volunteers were randomly divided into 2 groups, male-female. Under the condition of continuous electrocardiogram monitoring and the use of constant speed pump, hydrochloric acid nifekalant for injection of 0.3 mg/kg or 0.4 mg/kg were pushed into intravenous within 5 minutes, respectively. 4ml venous blood samples were obtained before and 5, 10, 15, 30, 45 min and 1, 2, 3, 4, 5, 6, 7 h after the administration of hydrochloric acid nifekalant injection preparations. The blood samples were centrifuged and plasma were collected and stored at -80 ℃ for analysis. After the dosing ecg monitoring for 2 hours.

8 healthy volunteers (male-female), under the condition of continuous electrocardiogram monitoring, the use of constant speed pump, hydrochloric acid nifekalant for injection of 0.4 mg/kg were pushed into intravenous within 5 minutes, then intravenous drip at 0.4 mg/kg/h velocity for 6 hours .4ml venous blood samples were obtained before, at the end of the push intravenous injection, 5, 15, 30, 45, 60 min, 2, 4, 6 h after the start of intravenous drip and 5, 10, 15, 30, 45 min, 1, 2, 3, 4, 5, 6 h after the end of administration of hydrochloric acid nifekalant injection. The blood samples were centrifuged and plasma were collected and stored at -80 ℃ for analysis. After the dosing ecg monitoring for 2 hours.

## 3. Results

**3.1 Plasma concentrations of Nifekalant in each group**

The average plasma concentrations of Nifekalant after intravenous push injection of 0.3 mg/kg or 0.4 mg/kg within 5 min were show in table 2. The average plasma concentrations of Nifekalant after intravenous push injection of 0.4 mg/kg within 5 min, then intravenous drip of 0.4 mg/kg/h for 6 h were show in table 3. The time-concentrations curves were show in figure 2 and figure 3.

*Table 2: The nifekalant time-plasma concentrations after intravenous push injection of 0.3 mg/kg and 0.4 mg/kg ($\bar{X}$ ±SD, n=16)*

| Time (h) | Concentration (ng/mL) | |
|---|---|---|
| | 0.3 mg/kg | 0.4 mg/kg |
| 0.08 | 230.95±54.02 | 358.615±73.98 |
| 0.17 | 158.02±39.85 | 213.16±38.35 |
| 0.25 | 112.05±25.41 | 175.79±29.95 |
| 0.5 | 90.71±26.08 | 133.83±26.18 |
| 0.75 | 69.87±24.70 | 106.81±21.69 |
| 1 | 49.54±17.10 | 78.12±18.20 |
| 2 | 26.04±7.89 | 38.28±13.47 |
| 3 | 16.94±6.98 | 22.74±4.86 |
| 4 | 11.24±4.11 | 14.19±1.54 |
| 5 | 6.94±2.60 | 8.83±1.41 |

*Table 3: The nifekalant time-plasma concentrations after intravenous infusion of 0.4 mg/kg 6 hours ($\bar{X}$ ±SD, n=8)*

| | Time (h) | Concentration (ng/mL) | | Time (h) | Concentration (ng/mL) |
|---|---|---|---|---|---|
| During the period of intravenous drip | 0.08 | 470.48±104.25 | After the end of intravenous drip | 6.08 | 254.28±64.44 |
| | 0.17 | 353.64±125.08 | | 6.17 | 237.60±58.31 |
| | 0.5 | 334.97±135.90 | | 6.25 | 221.29±68.16 |
| | 0.75 | 323.24±115.51 | | 6.5 | 180.89±53.71 |
| | 1 | 309.42±115.55 | | 6.75 | 126.64±27.73 |
| | 2 | 369.80±95.80 | | 7 | 98.45±31.73 |
| | 4 | 423.80±86.40 | | 8 | 59.15±18.55 |
| | 6 | 344.69±96.17 | | 9 | 42.23±17.75 |
| | | | | 10 | 24.47±4.67 |
| | | | | 11 | 15.26±3.78 |
| | | | | 12 | 9.60±2.91 |

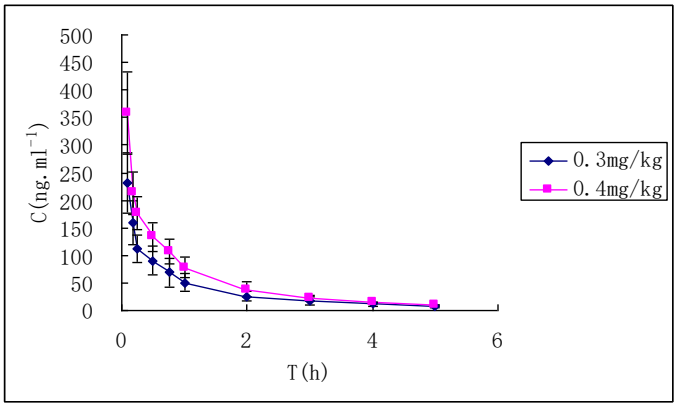


*Figure 2: The nifekalant time-plasma concentration curves after intravenous injection of 0.3 mg/kg and 0.4 mg/kg ($\bar{X}$ ±SD, n=16)*

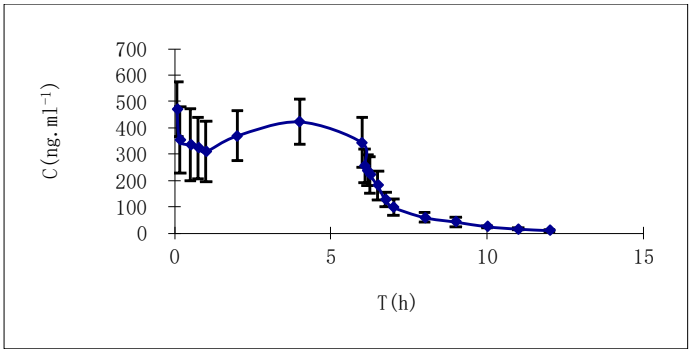


*Figure 3: The nifekalant time-plasma concentration curve after 6 hours intravenous infusion of 0.4 mg/kg ($\bar{X}$ ±SD, n=8)*

**3.2 Pharmacokinetic parameters of nifekalant in each group**

The mean pharmacokinetic parameters of nifekalant in each group after were shown in Tab 3 and Tab 4. The main pharmacokinetic parameters fitting by DAS2.0 was the second chamber model. There were not statistical differences of pharmacokinetic parameters between the two groups.

*Table 4: The nifekalant pharmacokinetic parameters after 5min intravenous injection of 0.3 mg/kg and 0.4 mg/kg and 6 hours intravenous infusion of 0.4 mg/kg ($\bar{X}$ ±SD, n=16)*

| parameters | A single dose intravenous push note | | Intravenous drug delivery |
|---|---|---|---|
| | 0.3 mg/kg | 0.4 mg/kg | 0.4 mg/kg |
| $t_{1/2}$ (h) | 1.54±0.38 | 1.34±0.19 | 1.35±0.23 |
| $T_{max}$ (h) | 0.08±0 | 0.08±0 | 3.75±1.28 |
| $C_{max}$ (ng/mL) | 230.95±54.02 | 358.62±73.98 | 444.30±88.12* |
| $AUC_{0-5}$ (ng/mL/h) | 193.53±45.19 | 285.61±46.57 | 2609.02±498.20 |
| AUC0-∞ (ng/mL/h) | 209.90±48.12 | 302.44±50.19 | 2627.33±499.89 |
| MRT (h) | 1.18±0.15 | 1.13±0.08 | - |
| CL (L/Kg/h) | 1.50±0.35 | 1.35±0.22 | 0.32±0.06 |
| V (L/Kg) | 3.40±1.44 | 2.60±0.35 | 0.61±0.15 |

## 4. Discussions

Nifekalant 14c markers rapidly distributed in other groups of thymus gland and seminal vesicle in rat after single dose of intravenous dosing, and the concentration in plasma and tissue increased with the same amount after 4 h (except the thymus), and the plasma radioactivity eliminated in three-phase, continuously dosing no accumulation occursed. The prototype of Nifekalant in dog blood eliminated with three phase after single dose of intravenous administration, the half-life of 0.47 ~ 0.72 h. The concentration of Nifekalant in blood by continuously dosing is the same as a single dosing. Plasma protein binding in rats and dogs were 65 ~ 70% and 68 ~ 80% respectively. The excretion rate of 14c markers in urine and dung (3:7) was more than 95% in the rat after single dose intravenous administration 24 h. The dog urine excretion rate was 46% and the fecal excretion rate was 27%. The above content was reported by Kondoh et al. (1994) and Martin et al. (1995). The mainly metabolic ways of Nifekalant are complexation with hydroxyethyl and N–removing the alkylation and the nitro reduction which were confirmed (Satoh et al. (2004) and Lee et al. (2004)). The radioactivity of 14c markers in breast milk was about 70 times than that of the plasma after Lactation rats intravenous 1h, which means Nifekalant transfering to milk height reported by Oyabe and Sano (2002).

Naitoh et al. (1998) and Kamiya et al. (1992) reported that the healthy male volunteers were participated in study, Nifekalant were pushed into intravenous, T1/2 was 1.53 ~ 2.07 h, About 86.4 ~ 94.6% Nifekalan was protein bound in serum, the main metabolites in plasma was glucuronic acid complex, rising rates of AUC and dosage were positively, the other parameters had nothing to do with the dosage. IN this study, hydrochloric acid nifekalant for injection of 0.3 mg/kg or 0.4 mg/kg were pushed into intravenous of healthy volunteers. Plasma drug concentration reached to the peak immediately after injection. The half-life was 1.54 hours and 1.34 hours respectively. Area under the curve (AUC) was positively related with dose. After 0.4 mg/kg intravenous pushing for 5 minutes, the injection of 0.4 mg/kg/h continuous intravenous drip, Nifekalant blood drug concentration rise steadily, and steady-state concentration after 4 hours, the steady state concentration of 423 ng. Ml-1. Slightly less than 543 ng.ml-1 reported by Tsuyoshi et al. (2009).

In this study, a HPLC method for determining the blood concentration of nifekalant with Ornidazole as internal standard in chinese people plasma was developed and reported. It is sensitivity, specialty and precision, and suitable for Nifekalant therapy drug monitoring and pharmacokinetic studies. The whole experiment process was smoothly, no adverse reaction occurred.

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