Journal of Chromatography, 617 (1993) 163–167
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6881

Short Communication

Sensitive method for determination of nicorandil in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection

Masahiko Tanikawa*, Megumi Uzu, Yasutsugu Ohsawa and Masafumi Fukushima

Assay Technology Laboratory, Development and Technology Division, Chugai Pharmaceutical Co. Ltd., 41-8 Takada 3-chome, Toshima-ku, Tokyo 171 (Japan)

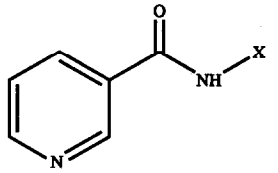
(First received January 15th, 1993; revised manuscript received April 6th, 1993)

ABSTRACT

A sensitive method for the determination of nicorandil, an anti-anginal drug, has been developed. The method involves solid-phase extraction of the drug and an internal standard using a Bond-Elut PH extraction column, liquid–liquid extraction with methyl acetate and dichloromethane, and reversed-phase high-performance liquid chromatography on a μBondapak C_{18} column with an ultraviolet detector. The limit of determination in plasma was 3 ng/ml and the intra-day coefficient of variation (n = 7) was less than 10%.

INTRODUCTION

Nicorandil, N-[2-(nitroxy)ethyl]-3-pyridinecarboxamide (SG-75), is a coronary vasodilator that is clinically used for the treatment of angina pectoris, especially effort–rest angina and variant angina caused by coronary spasms [1–6]. Infusions of nicorandil for the treatment of unstable angina or brain spasms are also under development [7]. The structures of nicorandil and its internal standard, N-[2-(nitroxy)propyl]-3-pyridinecarboxamide (SG-89), are shown in Fig. 1.



X = $(CH_2)_2ONO_2$: Nicorandil (SG-75)

= $(CH_2)_3ONO_2$: Internal standard (SG-89)

Fig. 1. Structures of nicorandil and an n-propyl homologue used as an internal standard.

In general, the determination of nicorandil has previously been performed by reversed-phase high-performance liquid chromatography (HPLC) after cartridge column extraction [8,9] or liquid–liquid extraction [10]; these methods have a detection limit of 10 ng/ml in human plasma. However, a more sensitive method was required in clinical studies. For this purpose, methods using a photoconductivity detector [9,11] or gas chromatography–mass spectrometry [12] have

* Corresponding author.

0378-4347/93/$06.00

been reported. Though these methods have high sensitivity (2 ng/ml and less than 1 ng/ml, respectively), the former requires a special system and the latter needs much time for sample preparation.

This paper describes a sensitive method for determining nicorandil in human plasma using reversed-phase HPLC with UV detection.

EXPERIMENTAL

Materials

Nicorandil and the internal standard (I.S.) were synthesized by Chugai Pharmaceutical (Tokyo, Japan). Water was purified with a Millipore Milli-Q water purification system (Bedford, MA, USA). All reagents were reagent grade and all solvents were HPLC grade.

Equipment

A Hitachi HPLC system (Hitachi, Tokyo, Japan) consisting of an L-6200 pump, an L-4000 UV detector and an AS-2000 autoinjector was used. The detector response was analysed using a D-2500 reporting integrator (Hitachi). A stainless-steel column packed with octadecylsilica (μ-Bondapak C_{18}, 300 mm × 3.9 mm I.D., 10 μm particle size, Japan Waters Assoc., Tokyo, Japan) was used. For protection of the analytical column, a precolumn filter (2 μm, GL Sciences, Tokyo, Japan) was used.

HPLC conditions

A degassed solution of 0.1 *M* borate buffer (pH 8)–water–acetonitrile (15:70:15, v/v) was used as the mobile phase. The detection wavelength was 220 nm. The flow-rate was 1.0 ml/min and room temperature at 25°C was maintained throughout analysis. The injection volume was 45 μl.

Sample preparation

To 500 μl of plasma sample, 50 μl of I.S. solution (2.5 μg/ml), *i.e.* 125 ng of the I.S., were added. The mixture was loaded on the Bond Elut PH column, which had been previously conditioned with 2 ml of methanol, followed by 2 ml of water. After passage of the plasma through the column, it was rinsed with 2 ml of water, followed by 1 ml of 8% methanol (v/v). Nicorandil and the I.S. were eluted into a centrifuge tube with 500 μl of 50% methanol (v/v). The eluted samples were evaporated to dryness under a stream of nitrogen at room temperature. This residue was taken up with 1 ml of water and acidified with 100 μl of 2 *M* hydrochloric acid. After the addition of 4 ml of ethyl acetate to the acid solution, the mixture was shaken for 10 min and then centrifuged at 2800 *g* for 10 min. The upper organic layer was removed, and the remaining aqueous layer was made alkaline (pH 9–10) with 300 μl of 1 *M* sodium carbonate. Then 4 ml of dichloromethane were added to the alkaline solution, and the mixture was shaken for 10 min. After centrifugation at 2800 *g* for 10 min, the dichloromethane layer was collected into a glass culture tube and evaporated to dryness under nitrogen at room temperature. The residue was dissolved in 100 μl of distilled water, and 45 μl of the solution were injected into the HPLC system.

Calibration curve

Standard solutions containing 30, 50, 100, 200, 500, 1000 and 2000 ng/ml nicorandil in water were prepared. Aliquots (50 μl) of these standard solutions were spiked into control human plasma (450 μl) in centrifuge tubes to give a calibration curve at concentrations of 3.0–200 ng/ml. These samples were treated as described above. The ratios of the peak height of nicorandil to that of the I.S. were used to construct the plasma calibration curve.

Validation

The intra-day ($n = 7$) and inter-day ($n = 3$) reproducibility were examined. A 1-ml volume of a standard solution containing 30, 100 or 1000 ng/ml nicorandil was added to 9.0 ml of control human plasma. These working samples (3.0, 10.0 and 100.0 ng/ml nicorandil in plasma) were stored at −20°C until analysis. The inter-day variability was assessed over three days using working samples. Similarly the intra-day variability was assessed with seven repeated measure-

ments for each of the three working samples on the same day. The nicorandil concentrations were calculated from the calibration curve run on the same day. Standard solutions were also injected to estimate the recovery from the peak height.

RESULTS AND DISCUSSION

Preparation

In previously reported methods for nicorandil, biological samples were pretreated by solid-phase extraction and analysed by HPLC with UV detection at 254 nm [8–10]. These methods were rapid and simple, but a highly sensitive quantification of nicorandil was required to determine the time course of this drug in clinical trials. However, it is impossible to develop a highly sensitive method with UV detection at 254 nm, because the molar absorption coefficient of nicorandil at 254 nm is 3500. At 220 nm, however, the molar absorption coefficient of nicorandil is twice that at 254 nm. Accordingly, we used a detection wavelength of 220 nm. Although this caused interference peaks originating from biological substances, these peaks were eliminated by liquid–liquid extraction with ethyl acetate and dichloromethane. A typical chromatogram is presented in Fig. 2.

Validation and application

The reproducibility and linear range of this method were examined using human plasma spiked with the standard solution of nicorandil. The limit of determination at a signal-to-noise ratio of 3 was 3 ng/ml. The calibration curve was

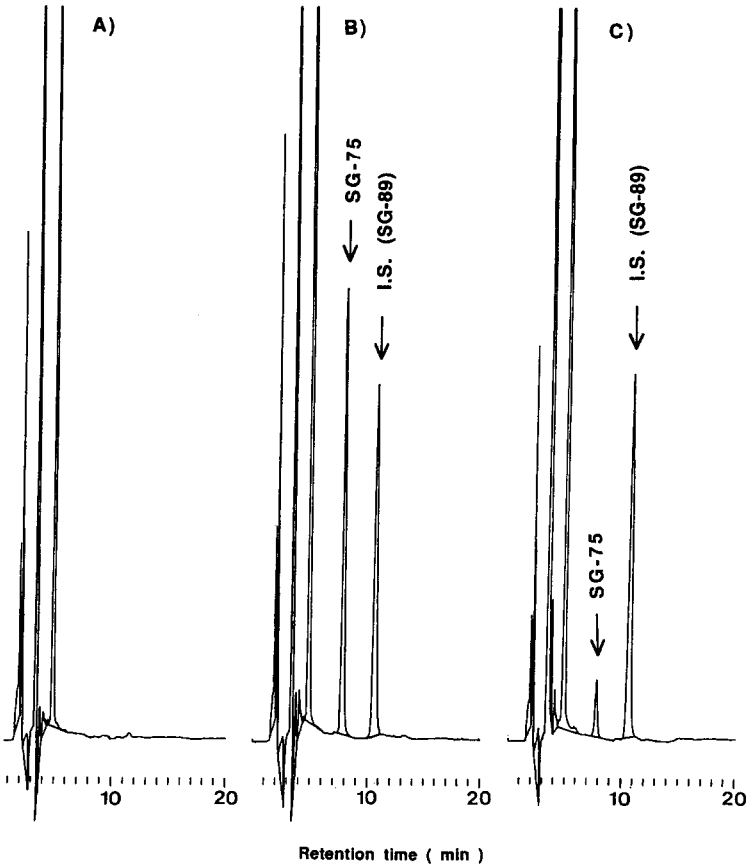


Fig. 2. Typical chromatograms for nicorandil (SG-75) and the internal standard (I.S.). (A) Drug-free human plasma; (B) spiked human plasma (nicorandil 200 ng/ml); (C) patient's plasma (30 min after administration of 4 mg of nicorandil).

TABLE I

RECOVERY OF NICORANDIL FROM SPIKED HUMAN PLASMA

Concentration added (ng/ml)	Recovery (mean ± S.D., n = 6) (%)
3.00	89.8 ± 17.8
5.00	87.5 ± 15.0
10.00	79.3 ± 7.4
20.00	78.9 ± 9.0
50.00	78.0 ± 6.5
100.00	78.5 ± 8.4
200.00	76.6 ± 9.1
Mean	81.2

TABLE II

INTRA-DAY AND INTER-DAY VARIATIONS IN THE DETERMINATION OF NICORANDIL IN HUMAN PLASMA

n	Nicorandil (ng/ml)			C.V. (%)
	Added	Measured	S.D.	
Intra-day				
7	3.00	3.35	0.35	10.3
7	10.00	9.32	0.67	7.2
7	100.00	101.88	1.41	1.4
Inter-day				
3	3.00	3.12	0.49	15.5
3	10.00	9.56	0.52	5.4
3	100.00	100.69	3.04	3.0

linear in the range from 3.0 to 200 ng/ml with correlation coefficients (r) of at least 0.9998. The recovery from the sample preparations was estimated from the ratio of the peak height of the plasma sample to that of the standard solution, and was in range 76.6–89.8%. These results are shown in Table I.

The intra-day coefficients of variation (C.V.) evaluated at concentrations of 3.0, 10.0 and 100.0 ng/ml were 10.3, 7.2 and 1.4%, respectively, and the inter-day C.V. at the same concentrations were 15.5, 5.4 and 3.0%, respectively. The results are shown in Table II.

Blood samples were collected 5, 15, 30, 60, 90, 120 and 180 min after intravenous administration of a 4-mg dose of nicorandil to a patient. The plasma concentration–time profile determined by this method is shown in Fig. 3. The maximum plasma concentration (C_{max}) of nicorandil was reached soon after administration, and was 118.8 ng/ml. Until the 60-min time interval, the plasma concentration curve declined very rapidly, and after that it showed a gradual decline. The nicorandil concentrations at 60, 120 and 180 min were 8.0, 3.3 and 2.6 ng/ml, respectively. From 60

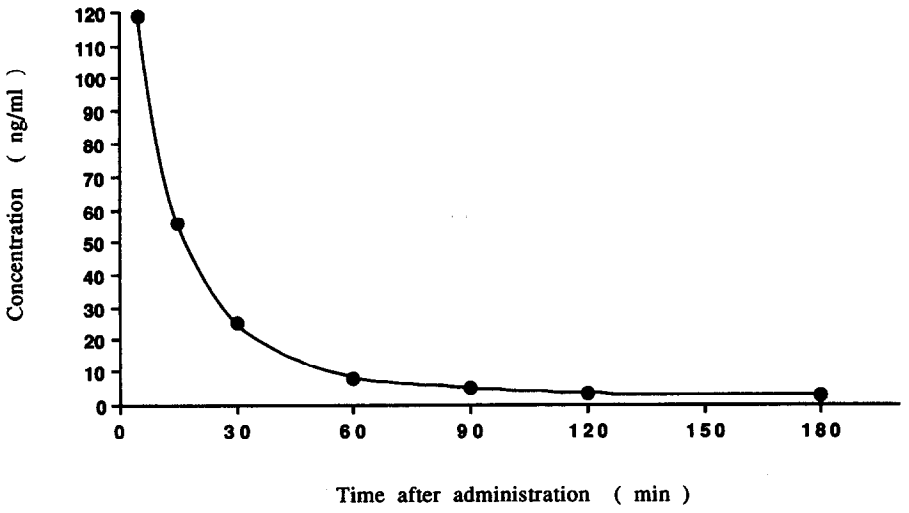


Fig. 3. Plasma nicorandil concentrations after intravenous administration of 4 mg of nicorandil to a patient.

min after administration, it was impossible to detect nicorandil using the published methods with absorbance detection at 254 nm. This newly developed assay method is the most sensitive of the reported methods using reversed-phase HPLC with UV detection, and so is useful for the determination of plasma nicorandil levels in clinical studies.

REFERENCES

1 Y. Uchida, N. Yoshimoto and S. Murano, *Jpn. Heart J.*, 19 (1978) 112.
2 Y. Nakagawa, K. Takeda, Y. Katano, T. Tsukada, T. Kitagawa, T. Otori and S. Imai, *Jpn. Heart J.*, 20 (1979) 881.
3 K. Sakai, Y. Shirai and H. Nabata, *J. Cardiovasc. Pharmacol.*, 3 (1981) 139.
4 H. Murao and E. Kimura, *Jpn. J. Clin. Pharmacol.*, 13 (1982) 311.
5 M. Kinoshita, S. Nishikawa, M. Sawamura, S. Yamaguchi, K. Jitsunami, M. Itoh, M. Motomura, K. Bito, I. Mashiro and S. Kawakita, *Am. J. Cardiol.*, 58 (1986) 733.
6 N. Taira, *J. Cardiovasc. Pharmacol.*, 10 (Suppl. 8) (1987) S1.
7 J. Azuma, H. Harada, Y. Seto, T. Yamamoto, R. Kaida, M. Abe, A. Okazaki, *Jpn. J. Pharmacol. Ther.*, 18 (1990) 3479.
8 F. J. Schwende and R. C. Lewis, *J. Chromatogr.*, 525 (1990) 151.
9 Y. Gomita, K. Furuno, K. Eto, T. Fukuda, Y. Araki, M. Yamori and R. Oishi, *J. Chromatogr.*, 528 (1990) 509.
10 T. Ishizaki, K. Chiba, T. Suganuma, T. Sasaki, H. Kamiyama and H. Nakano, *J. Pharm. Sci.*, 73 (1984) 494.
11 D. J. Popovich, J. B. Dixon and B. J. Ehrlich, *J. Chromatogr. Sci.*, 17 (1979) 643.
12 A. M. Frydman, P. Chapelle, H. Diekmann, R. Bruno, J. J. Thebault, J. Bouthier, H. Caplain, W. Ungethuem, C. Gaillard, A. L. Liboux, A. Renard and J. Gaillot, *Am. J. Cardiol.*, 63 (1989) 25j.