*Journal of Chromatography*, 592 (1992) 317–322
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2461

# Determination of the enantiomers of fenoldopam in human plasma by reversed-phase high-performance liquid chromatography after chiral derivatization

Venkata K. Boppana*, Lauren Geschwindt, Matthew J. Cyronak and Gerald Rhodes

*Department of Drug Metabolism and Pharmacokinetics, SmithKline Beecham Pharmaceuticals, P.O. Box 1539, Mail Code L-712, King of Prussia, PA 19406 (USA)*

## ABSTRACT

Fenoldopam, a selective agonist at peripheral dopaminergic (DA-1) receptors, is administered as a racemic mixture and, consequently, an indirect stereospecific high-performance liquid chromatographic assay was developed to study the disposition of the individual enantiomers in human subjects. Fenoldopam enantiomers were extracted from alkalinized plasma into ethyl acetate prior to precolumn derivatization with the chiral reagent 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC). The resulting diastereomers were separated on a reveresed-phase butylsilica column and determined using triple-electrode coulometric detection. The limits of determination and detection for the *S*- and *R*-enantiomers of fenoldopam were 0.5 and 0.25 ng/ml, respectively. A linear response was observed for (*S*)- and (*R*)-fenoldopam concentrations ranging from 0.5 to 50 ng/ml in plasma. The intra-day relative standard deviations (R.S.D.s) for the plasma assay at nominal concentrations of 0.5, 5 and 50 ng/ml were 17.4, 5.2 and 6.9%, respectively, for (*S*)-fenoldopam and 9.9, 6.2 and 7.4%, respectively, for (*R*)-fenoldopam. The inter-day R.S.D.s of the method at these concentrations were 9.3, 7.7 and 7.4%, respectively, for (*S*)-fenoldopam and 9.5, 1.9 and 7.3%, respectively, for (*R*)-fenoldopam. The mean accuracy of the method at concentrations of 0.5, 5 and 50 ng/ml in plasma was found to be 106.4, 111.8 and 108.9%, respectively, for (*S*)-fenoldopam and 116.2, 104.2 and 111.2%, respectively, for (*R*)-fenoldopam. The assay developed was sufficiently sensitive, accurate and precise to support pharmacokinetic studies in human subjects.

## INTRODUCTION

Fenoldopam (SK&F 82526, **I**, Fig. 1) [6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1*H*-3-benzazepine-7,8-diol], a potent agonist at peripheral dopamine-1 (DA-1) receptors, has been shown to produce beneficial effects including an improvement in renal blood flow and a reduction in blood pressure following intravenous and oral administration [1,2]. Fenoldopam contains a chiral center and exists in two enantiomeric forms (*S* and *R*), of which only the *R*-enantiomer exhibits the desired pharmacological activity [3]. As fenoldopam is administered as the racemate, a stereospecific assay method was required in order to study the disposition of the individual enantiomers in human subjects.

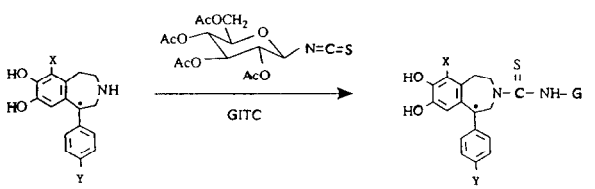


Fig. 1. Derivatization of fenoldopam (X = Cl; Y = OH) and internal standard (SK&F 38393, X = Y = H) with GITC to form the corresponding diastereomeric thiourea products. Ac = Acetyl.

Previously described methods based on high-performance liquid chromatography (HPLC) with electrochemical detection (ED) for the measurement of racemic fenoldopam and its metabolites [4–7] in various biological fluids were not readily modifiable for the direct measurement of (*S*)- and (*R*)-fenoldopam in plasma. Consequently, this paper describes an indirect stereospecific HPLC–ED method for the simultaneous determination of the

0021-9673/92/$05.00 

individual enantiomers of fenoldopam in human plasma.

## EXPERIMENTAL

### *Chemicals and supplies*

Fenoldopam mesylate (racemic mixture), internal standard (racemic SK&F 38393-A, I.S., Fig. 1) and the mesylate salts of (*R*)- and (*S*)-fenoldopam were obtained from Drug Substances and Products, SmithKline Beecham Pharmaceuticals (Swedeland, PA, USA). Dibasic sodium phosphate and HPLC-grade methanol, acetonitrile and ethyl acetate were obtained from J.T. Baker (Phillipsburg, NJ, USA). The chiral reagent 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) was purchased from Polysciences (Warrington, PA, USA). Glacial acetic acid was obtained from Mallinckrodt (Paris, KY, USA). HPLC-grade water (Milli-Q water purification system; Millipore, Bedford, MA, USA) was used in the preparation of buffer and reagent solutions. All other chemicals were of analytical-reagent grade. Microfilterfuge centrifugal filter tubes, with 0.45-μm pore size nylon 66 membrane, were obtained from Rainin Instruments (Woburn, MA, USA).

### *Standard solutions and reagents*

Stock standard solutions of fenoldopam and internal standard were prepared separately by dissolving 13.14 mg of fenoldopam mesylate and 11.4 mg of SK&F 38393 (hydrochloride salt) in 0.05 *M* acetic acid in a 10-ml volumetric flask to give a final concentration of 1 mg/ml. Appropriate dilutions of the stock solutions of fenoldopam were made with 0.05 *M* acetic acid to generate a series of working standard solutions (100, 10, 1, 0.1 and 0.01 μg/ml). All stock and working standard solutions were stable for 4 weeks when stored at 4°C. Freshly prepared ascorbic acid solution in water was added to plasma to give a final concentration of 0.5% (w/v). Samples were protected from light.

*GITC reagent solution (200 μg/ml).* A 2 mg/ml solution of GITC in ethyl acetate was prepared by dissolving a weighed amount of GITC in an appropriate volume of ethyl acetate. This was further diluted with ethyl acetate to give a final concentration of 200 μg/ml. This solution was prepared fresh daily just before use.

*Citrate–acetate buffer (pH 5.6).* A 22.0-g amount of sodium acetate trihydrate, 21 g of citric acid monohydrate, 9.8 g of sodium hydroxide and 0.63 g of disodium EDTA were dissolved in 2 l of water. The ionic strength and pH of the solution were 260 m*M* and 5.6, respectively.

*Mobile phase.* A 75-ml volume of methanol, 85 ml of acetonitrile, 140 ml of citrate–acetate buffer (pH 5.6) and 210 ml of water were mixed and filtered through a Millipore 0.45-μm type HA membrane filter. The mobile phase was recycled for a period of 7 days.

*Calibration.* A set of 1-ml plasma calibration standards [concentrations of (*S*)- and (*R*)-fenoldopam of 0.25, 0.5, 1, 2, 5, 10, 20 and 50 ng/ml] was prepared from working standard solutions of fenoldopam and analyzed with each determination of fenoldopam enantiomer plasma concentrations. The peak-height ratios of (*S*)- and (*R*)-fenoldopam to the internal standard were weighted by $1/y$ (based on analysis of residual plots) and plotted against analyte concentration for both (*S*)- and (*R*)-fenoldopam. Linear regression analysis gave calibration lines that were used to calculate the concentrations of (*S*)- and (*R*)-fenoldopam in spiked control or unknown plasma samples.

### *Collection of clinical samples*

Ten healthy male volunteers received an intravenous infusion of fenoldopam at a rate of 1 μg/kg for 2 h. Blood samples were collected at 0, 5, 15, 30, 45, 60, 90, 120, 122.5, 125, 130, 135, 140, 150, 165, 180, 210, 240, 270, 300, 330 and 360 min in heparinized Vacutainers and centrifuged at 3000 *g*. Samples of 4.75 ml of plasma were transferred into 100 × 17 mm I.D. polypropylene tubes containing 0.25 ml of 10% ascorbic acid (freshly prepared), mixed and stored immediately at −20°C. Addition of ascorbic acid is essential to ensure stability of fenoldopam in plasma during the storage in the freezer.

### *Extraction procedure*

An aliquot of plasma (1 ml), containing fenoldopam as standard or as an unknown, was mixed with 50 μl of working internal standard (I.S.) solution (500 ng/ml) in a 100 × 13 mm I.D. screw-capped Pyrex glass tube. Ethyl acetate (5 ml) was added to the tube, followed by 0.5 m*M* of dibasic sodium phosphate solution (0.5 ml). After mixing the con-

tents by inverting the tube, the latter was placed on a reciprocal shaker and the contents were mixed at low speed (60 cycles/min) for 10 min. Following centrifugation of the sample tube at 2000 *g* for 10 min, the ethyl acetate fraction (4.5 ml) was transferred into a 100 × 13 mm I.D. borosilicate tube and the solvent was evaporated under nitrogen at 40°C. The dried samples was then subjected to derivatization with GITC.

*Derivatization procedure*

To the dried extract, 100 μl of GITC solution (200 μg/ml) were added followed by 50 μl of 1% triethylamine (freshly prepared) in ethyl acetate. The sample was allowed to stand at room temperature for 1 h. Following reaction, the solvent was removed under a stream of nitrogen at 40°C and the residue was reconstituted in 200 μl of 0.05 *M* acetic acid containing 30% (v/v) of acetonitrile. The sample was filtered through a microfilterfuge centrifugal filter tube (0.45-μm pore size, nylon 66 membrane) that had been previously washed with 0.5 ml of 0.05 *M* acetic acid containing 30% (v/v) of acetonitrile. The filtrate was transferred into an autosampler vial and 5–50 μl were injected for HPLC analysis.

Optimization of the precolumn reaction proceeded from the initial conditions described above using a standard solution of racemic fenoldopam. This standard solution was repetitively derivatized using different reagent concentrations and reaction times prior to HPLC separation and analysis. The coulometric response for each diastereomer was monitored by measuring the resulting peak height while keeping the HPLC conditions constant. Using this approach, optimum conditions were determined for maximum coulometric response following precolumn derivatization. The reaction was also monitored for complete conversion of substrate (fenoldopam) to the respective GITC derivatives by measuring the racemic fenoldopam levels in the reaction media according to the method published earlier [4].

*High-performance liquid chromatography*

The isocratic chromatographic system consisted of an HPLC pump (Model 114; Beckman Instruments, Palo Alto, CA, USA) and an autoinjector (WISP Model 710B; Waters Assoc., Milford, MA, USA). Separations were carried out on an Aquapore butylsilica (7 μm) column (22 cm × 2.1 mm I.D.) (Pierce, Rockford, IL, USA) coupled in-line with a butylsilica guard column (3 cm × 2.1 mm I.D.) (Pierce). The column temperature was maintained at 37°C using a column heater (Rainin Instruments). The mobile phase was pumped at a flow-rate of 0.3 ml/min. Prior to use, the mobile phase was degassed by filtration through a 0.45-μm membrane filter. Following chromatographic separation, the analytes were detected by oxidation of the catechol moiety with a coulometric detector (ESA, Bedford, MA, USA) equipped with three serial electrodes (one guard, G, and two analytical cells, $W_1$ and $W_2$). The potential used at these electrodes were G = +0.2 V, $W_1$ = −0.20 V and $W_2$ = +0.20 V. Chromatographic peak-height data were collected with a computerized automated laboratory system (Access*Chrom; PE/Nelson, Cupertino, CA, USA).

*Validation procedures*

Three pools of plasma precision samples containing 0.5, 5 and 50 ng/ml each of (*S*)- and (*R*)-fenoldopam were prepared by adding appropriate volumes of standard solutions to drug-free plasma preserved with ascorbic acid (total volume 25 ml). These plasma samples were stored at −20°C until analysis. Five replicate samples from each pool were extracted and analyzed on three separate days. Concentrations were determined by comparison with a calibration graph prepared on the day of analysis. From the data obtained, intra- and inter-day relative standard deviations (R.S.D.s) and mean accuracies were calculated.

## RESULTS AND DISCUSSION

Initial efforts directed toward the development of a direct HPLC separation of (*S*)- and (*R*)-fenoldopam compatible with electrochemical detection were not successful. Consequently, an indirect method of analysis was pursued based upon precolumn chiral derivatization followed by chromatographic separation of the diastereomeric products formed. Of the several chiral reagents commercially available, GITC is widely used for precolumn derivatization of primary and secondary amines [8–11] to develop stereospecific HPLC methods for enantiomeric compounds. GITC reacts rapidly with

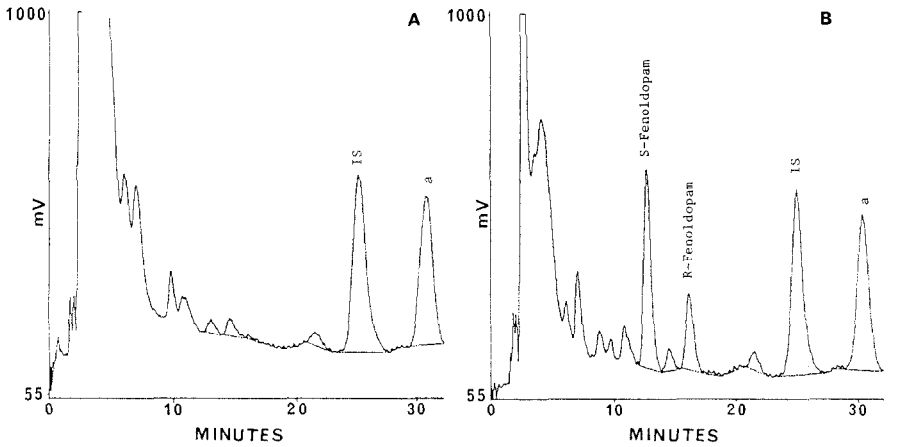


Fig. 2. Chromatogram of plasma extracts from a human subject (A) before and (B) 2 h after intravenous infusion of 1 μg/min · kg of racemic fenoldopam for 2 h. The plasma concentrations of (*S*)- and (*R*)-fenoldopam were 10.06 and 4.95 ng/ml, respectively. The peak labeled a is the other diastereomer of the internal standard. For chromatographic conditions, see the text.

primary and secondary amines, it is commercially available in highly pure form and it is stereochemically stable. The isothiocyanate group of GTIC reacts selectively with secondary amine function of fenoldopam under mild reaction conditions to form corresponding stable thiourea derivative of fenoldopam (Fig. 1). Structures of the derivatives were confirmed by fast atom bombardment mass spectrometry.

The stereospecific plasma assay described here for the determination of (*S*)- and (*R*)-fenoldopam involved ethyl acetate extraction of the drug and the internal standard from alkalinized plasma as a preliminary isolation step, followed by derivatization of the individual enantiomers with GITC. Derivatization of fenoldopam with GITC was carried out in various solvents, such as dimethylformamide, acetonitrile, chloroform and ethyl acetate, with satisfactory results. Ethyl acetate was chosen as the solvent to conduct the derivatization reaction as a higher rate of reaction was observed and, moreover, it was used as the extractions solvent for the isolation of fenoldopam enantiomers from plasma. To facilitate derivatization, a small amount of triethylamine was also added to the reaction media. Further optimization of the precolumn derivatization of fenoldopam with GITC was carried out in ethyl acetate at room temperature by varying the concentration of GITC (20–2000 μg/ml) and reaction time (30–120 min). The resultes indicated that a GITC concentration of 200 μg/ml and a reaction time of 60 min gave the maximum coulometric response for fenoldopam enantiomers. The diastereomeric thiourea derivatives formed were then analyzed by reversed-phase HPLC–ED. Coulometric detection utilized three electrodes in series so that the catechol moiety of the derivatized analyte would undergo a series of oxidation and reduction reactions (oxidation, reduction, oxidation), with final measurement in the oxidized form. These series of electrochemical reactions can provide additional selectivity for compounds which undergo reversible electrochemical reactions. The use of this technique was necessary in this instance to remove a large negative signal arising from excess of reagent that eluted near the analytes, and to increase the overall sensitivity of detection. The use of narrow-bore HPLC columns (2 mm I.D.) also provided enhanced sensitivity for the detection of GITC-derivatized (*S*)- and (*R*)-fenoldopam.

Typical chromatograms of extracts from a drug-free human plasma sample and a plasma sample obtained from a human subject who had received racemic fenoldopam by intravenous infusion are shown in Fig. 2. The diastereomeric products were well resolved and the retention times were highly reproducible. The assignment of the chromatographic peaks as the diastereomers of (*S*)- and (*R*)-

fenoldopam was accomplished by separate GITC derivatization and chromatographic analysis of standard solutions of the pure *S*- and *R*-enantiomers. Endogenous plasma components did not interfere with the quantification of (*S*)- and (*R*)-fenoldopam or the internal standard over the plasma concentration range 0.5–50 ng/ml. The recoveries of fenoldopam and the internal standard from plasma was determined previously using the racemic compounds and found to be 89.7 ± 2.96% and 93.4 ± 5.95%, respectively [5]. Recovery of the individual *S*- and *R*-enantiomers of fenoldopam from plasma was not conducted owing to non-availability of the pure diastereomers. As the reaction of fenoldopam with GITC was essentially complete, as judged by the disappearance of fenoldopam from the reaction medium, the recovery through the total method was also high. Using 1 ml of plasma, the limit of determination for both (*S*)- and (*R*)-fenoldopam in human plasma samples was 0.5 ng/ml (signal-to-noise ratio = 4). There was, however, an interfering chromatographic signal which overlapped as a trailing shoulder on the (*S*)-fenoldopam peak, and which prevented determination at levels below 0.5 ng/ml. Linear responses of the peak-height ratio of (*S*)- and (*R*)-fenoldopam to the internal standard were observed with concentrations of analyte ranging from 0.5 to 50 ng/ml. Correlation coefficients obtained using weighted ($1/y$) linear regression analysis were typically 0.999.

Results of a 3-day validation study are given in Table I for both enantiomers. The intra-day precision of the method was indicated by the mean of the daily R.S.D. The inter-day precision of the method was indicated by the R.S.D. of the daily means. The inter-day R.S.D.s of the method were also calculated by analysing two pools of quality control plasma samples spiked with 2 and 20 ng/ml of each of (*S*)- and (*R*)-fenoldopam over a period of 12 days. The inter-day R.S.D.s, from the analysis of these samples, were found to be 12.1 and 11.7% for (*S*)-fenoldopam and 16.8 and 11.7% for (*R*)-fenoldopam, respectively. The mean accuracy of the method, as indicated by the ratio of the actual to theoretical concentration, is also shown in Table I.

In conclusion, we have described a sensitive HPLC–ED method for the determination of (*S*)- and (*R*)-fenoldopam in human plasma samples. The limit of determination using 1-ml plasma samples was 0.5 ng/ml for each individual enantiomer. The assay was linear in response over the range 0.5–50 ng/ml. The method was sufficiently sensitive, accu-

TABLE I

ACCURACY AND PRECISION DATA FOR (*S*)- AND (*R*)-FENOLDOPAM IN PLASMA

| Parameter | Concentration in plasma (ng/ml) | | | | | |
|---|---|---|---|---|---|---|
| | 0.5 ng/ml | | 5 ng/ml | | 50 ng/ml | |
| | (*S*) | (*R*) | (*S*) | (*R* | (*S*) | (*R*) |
| R.S.D. (%): | | | | | | |
| Day 1 | 16.5 | 5.7 | 3.0 | 6.1 | 7.1 | 6.7 |
| Day 2 | 15.9 | 17.9 | 4.5 | 4.8 | 6.8 | 8.8 |
| Day 3 | 19.8 | 6.2 | 8.0 | 7.7 | 6.9 | 6.8 |
| Error (%)[a]: | | | | | | |
| Day 1 | −4.0 | +26,0 | +12.4 | +14.0 | +10.9 | +8.5 |
| Day 2 | +14.0 | +4.0 | +25.8 | +14.8 | −4.4 | −5.3 |
| Day 3 | +14.0 | +16.0 | +8.8 | +10.8 | +3.9 | +6.8 |
| Inter-day R.S.D. (%)[b] | 9.3 | 9.5 | 7.7 | 1.9 | 7.4 | 7.3 |
| Intra-day R.S.D. (%)[c] | 17.4 | 9.9 | 5.2 | 6.2 | 6.9 | 7.4 |
| Mean accuracy (%) | 106.4 | 116.2 | 111.8 | 104.2 | 108.9 | 111.2 |

[a] [(Calculated concentration − actual concentration)/actual concentration] × 100.
[b] R.S.D. values of daily means.
[c] Mean of the daily R.S.D. values.

rate and precise to support pharmacokinetic studies on (*S*)- and (*R*)-fenoldopam in human subjects. Using this method, plasma concentrations of (*S*)- and (*R*)-fenoldopam could be measured for up to 4 h following intravenous administration of racemic fenoldopam, sufficient to characterize the intravenous pharmacokinetics of fenoldopam diastereomers.

## REFERENCES


1 R. M. Stote, J. W. Dubb, R. G. Familiar, B. B. Erb and F. Alexander, *Clin. Pharmacol. Ther.*, 34 (1983) 309.
2 H. O. Ventura, F. H. Messerli, E. D. Frohlich, I. Kobrin, W. Oigman, F. G. Dunn and R. M. Carey, *Circulation*, 69 (1984) 11142.
3 D. M. Ackerman, A. L. Blumberg, J. P. McMafferty, S. S. Sherman, J. Weinstock, C. Kaiser and B. Berkowitz, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 42 (1983) 186.
4 V. K. Boppana, F. C. Heineman, R. K. Lynn, W. C. Randolph and J. A. Ziemniak, *J. Chromatogr.*, 317 (1984) 463.
5 V. K. Boppana, K. M. Dolce, M. J. Cyronak and J. A. Ziemniak, *J. Chromatogr.*, 487 (1989) 385.
6 V. K. Boppana, K. L. Fong, J. A. Ziemniak and R. K. Lynn, *J. Chromatogr.*, 353 (1986) 231.
7 V. K. Boppana, R. K. Lynn and J. A. Ziemniak, *J. Pharm. Sci.*, 78 (1989) 127.
8 N. Nimura, H. Ogura and T. Kinoshita, *J. Chromatogr.*, 202 (1980) 375.
9 A. J. Sedman and J. Gal, *J. Chromatogr.*, 278 (1983) 199.
10 N. Nimura, Y. Kasahara and T. Kinoshita, *J. Chromatogr.*, 213 (1981) 327.
11 T. Kinoshita, Y. Kasahara and N. Nimura, *J. Chromatogr.*, 210 (1981) 77.