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DETERMINATION OF FENOLDOPAM (SK&F 82526) AND ITS METABOLITES IN HUMAN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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SUMMARY

Fenoldopam [6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1H-3-benzazepine-7,8-diol] is a potent renal vasodilator that is currently undergoing Phase II clinical trials. Quantitative analytical methods, based on high-performance liquid chromatography with electrochemical detection (HPLC–ED) after ethyl acetate extraction from plasma or urine were developed for the determination of fenoldopam and its identified metabolites in biological media. The lower limit of quantitation for fenoldopam in plasma was 50 pg/ml. In assays for fenoldopam glucuronide(s) and fenoldopam conjugates, urine was treated with β-glucuronidase and Glusulase, respectively, and the liberated fenoldopam was quantified by HPLC-ED. A novel assay by dual-electrode (in series) HPLC-ED was developed for the 8-sulfate of fenoldopam. In this method, the 8-sulfate was oxidized to the *o*-quinone at the first electrode and quantitated at the second electrode after reduction to the catechol. A similar dual-electrode HPLC–ED method was used for 7- and 8-O-methyl fenoldopam. Conjugates of the O-methyl metabolites were determined by HPLC–ED after hydrolysis to O-methyl fenoldopam. These methods have been used to study the kinetics and metabolism of fenoldopam in healthy volunteers. The methods are specific, sensitive, reproducible, and linear over a wide range of concentrations. Precision of the analyses, expressed as coefficients of variation, were less than 10% for all analyses.

INTRODUCTION

Fenoldopam (SK&F 82526) [6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1H-3-benzazepine-7,8-diol] (Fig. 1) is an orally active renal vasodilator in dogs, monkeys, hypertensive rats, and man[1–3]. Initial animal studies showed that, after oral administration of fenoldopam, the drug was rapidly metabolized and eliminated from the body. The catechol moiety of fenoldopam which is the principal site for metabolism is conjugated with sulfate to form the isomeric 7- and 8-sulfates (7-SO_4; 8-SO_4). Glucuronidation occurs exclusively at the 7-position (7-GLU)[4]. Minor metab-

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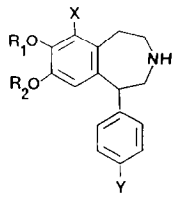


Fig. 1. Structures of fenoldopam, its metabolites and internal standards.

	R_1	R_2	X	Y
Fenoldopam	H	H	Cl	OH
8-SO_4	H	SO_3^-	Cl	OH
7-SO_4	SO_3^-	H	Cl	OH
7-GLU	$C_6H_9O_6$	H	Cl	OH
7-MEO	CH_3	H	Cl	OH
8-MEO	H	CH_3	Cl	OH
4'-SO_4	H	H	Cl	OSO_3^-
IS-A*	H	H	H	H
IS-B*	SO_3^-	H	H	H
IS-C*	H	CH_3	H	H

* Internal standards.

olites are the 7-O-methyl (7-MEO) and 8-O-methyl (8-MEO) analogues which are excreted in urine primarily as conjugates.

Pharmacokinetic and disposition studies of fenoldopam require sensitive and specific analytical methods for measuring the low concentrations of fenoldopam and its metabolites in plasma and urine. A previous analytical report described only a method for fenoldopam in plasma and did not present stability information for the compound in biological media[5]. The present paper describes improved analytical methods for measuring fenoldopam in plasma and fenoldopam and its glucuronide metabolites in urine. It also describes novel methods based on dual-electrode high-performance liquid chromatography with electrochemical detection (HPLC–ED) for direct measurement of the 8-SO_4 in plasma and urine and of the methoxy metabolites in urine.

MATERIALS AND METHODS

Chemicals

Fenoldopam, its metabolites and the internal standards were obtained from Drug Substances and Products, SK&F Laboratories. Methanol, acetonitrile, and ethyl acetate were HPLC-grade solvents (Fisher Scientific, Pittsburgh, PA, U.S.A.). Analytical-grade anhydrous diethyl ether, without preservative, was used (Mallinckrodt, Paris, KY, U.S.A.). All other chemicals were of analytical grade and were obtained from commercial suppliers. β-Glucuronidase (type B-1, from bovine liver) was obtained from Sigma (St. Louis, MO, U.S.A.) and Glusulase® (from *Helix pomatia*) was obtained from Endo Laboratories (Garden City, NY, U.S.A.).

Reagents

Enzyme solutions. Final activities of enzymes (prepared or diluted with water)

were: β-glucuronidase, 5000 units/ml; Glusulase, 25,000 units β-glucuronidase/ml and 5000 units sulfatase/ml.

Citrate–acetate buffer, pH 4.0. Sodium acetate trihydrate (11.0 g), citric acid monohydrate (10.5 g), sodium hydroxide (4.9 g), disodium EDTA (0.335 g) and acetic acid (37.5 ml) were dissolved in 1 l of deionized water.

Monochloroacetate buffer, pH 3.2. Monochloroacetic acid (28.3 g), sodium hydroxide (9.8 g), and disodium EDTA (1 g) were dissolved in 2 l of deionized water.

Mobile phases

(A) Methanol–citrate–acetate buffer, pH 4.0 (20:80); (B) methanol–monochloroacetate buffer, pH 3.2 (18:82); (C) acetonitrile–methanol–monochloroacetate buffer, pH 3.2 (18:4:78).

Standard solutions

Stock standard solutions (1 mg/ml solution for fenoldopam or 230 μg/ml for metabolites) were prepared in 0.05 *M* acetic acid, except for 8-SO_4 and its internal standard (IS-B), which were first dissolved in 2 ml methanol and then made up to volume with 0.05 *M* acetic acid. Appropriate dilutions of the stock solutions were made with 0.05 *M* acetic acid. All stock and working standard solutions were stored at 4°C, except for the stock solutions of 8-SO_4 and IS-B, which were stored at room temperature, because crystallization occurs at refrigerator temperatures. Freshly prepared ascorbic acid was added to plasma and urine (when making standards) to give a final concentration of 0.5%, w/v. Samples were protected from direct light.

Collection of clinical samples

A healthy male volunteer received an oral dose of 100 mg fenoldopam mesylate (free base). Blood samples were collected in heparinized Vacutainers and centrifuged at 3000 *g*. Samples of 4.75 ml plasma were transferred to 100 × 17 mm polypropylene tubes, containing 0.25 ml of 10% ascorbic acid (freshly prepared), mixed, and stored immediately at −20°C. The urine was collected in plastic bottles, containing ascorbic acid (to give an approximate final concentration of 0.5%, w/v); the volume was measured and aliquots were stored at −20°C.

Extraction procedures

Extraction of fenoldopam from plasma. A volume of 2 ml plasma, 50 μl of 0.05 *M* acetic acid (containing standards when preparing standard curves), and 50 μl internal standard (200 ng/ml, IS-A) were pipetted into a 100 × 17 mm polypropylene tube and thoroughly mixed. Ethyl acetate (5 ml) and 1.0 *M* sodium hydroxide (26 μl) were then added, and the tube was shaken for 10 min on a mechanical shaker at 60 cycles/min. The phases were separated by centrifugation at 1500 *g* for 10 min and 4.5 ml of the organic layer was transferred to another polypropylene tube. The ethyl acetate was evaporated under nitrogen at 40°C and the residue was dissolved in citrate–acetate buffer (pH 4.0, 300 μl) and diethyl ether (2 ml) added. The tubes were shaken for 5 min at 120 cycles/min on a mechanical shaker and centrifuged at 1500 *g* for 5 min. The ether layer was carefully aspirated and the tubes were placed under a nitrogen stream for 15 min to remove the remaining ether. The aqueous layer was transferred to autosampler vials, and 20–100 μl of the extract was injected into the liquid chromatograph.

Extraction of fenoldopam from urine. For measurement of unconjugated fenoldopam, 100 μl urine, 900 μl water, 50 μl of 0.05 *M* acetic acid (containing standards, when preparing standard curve) and 50-μl internal standard (500 ng/ml, IS-A) were thoroughly mixed in a 100 × 17 mm polypropylene tube. Ethyl acetate (2 ml) and 1.0 *M* phosphate buffer (pH 7.5, 200 μl) were then added. After mechanical shaking for 10 min and centrifugation for 10 min at 1500 *g*, 1.5 ml of the organic phase was transferred to another tube and evaporated to dryness under a nitrogen stream at 40°C. The rest of the procedure was the same as for plasma (see above). Injection volumes were 10–50 μl.

Hydrolysis of fenoldopam conjugates in urine. Two methods were developed, one for "total conjugates" and one which was specific for glucuronides. For total conjugates, 1 ml of urine was mixed with 1 ml 0.2 *M* acetate (pH 4.7) and 100 μl diluted Glusulase. For glucuronides, 1 ml urine, 1 ml 0.2 *M* phosphate (pH 4.7), and 100 μl β-glucuronidase were combined. After incubation at 37°C for 16 h, 7.9 ml water was added to each sample and 100 μl of diluted sample was extracted as described for fenoldopam in urine (see above).

Extraction of 8-SO_4 from plasma. C_{18} cartridges (Sep-Pak®, Waters Assoc.) were prepared by passing them through successively, 10 ml methanol, 20 ml water, and 2 ml phosphate buffer (1.0 *M*, pH 7.5). A volume of 1 ml plasma, 100 μl of 0.05 *M* acetic acid (containing standards when preparing standard curve), 50 μl internal standard (1.17 μg/ml, IS-B), and 1 ml phosphate buffer (1.0 *M*, pH 7.5) were mixed in a 100 × 17 mm polypropylene tube. The sample was then poured into a syringe barrel attached to a previously conditioned Sep-Pak cartridge and vacuum was applied. After passage of the sample through the cartridge, the packing was rinsed with 10 ml water and the cartridge was centrifuged to remove remaining traces of water. The cartridge was eluted with 2 ml of 0.3 *M* acetic acid in methanol. The methanol was evaporated at 40°C with a gentle stream of nitrogen. The residue was dissolved in 200 μl mobile phase B. Since the residue was not completely soluble, mechanical means (scraping) were employed to aid in dissolution of 8-SO_4. Following centrifugation of samples, the supernatants were transferred to autosampler vials, and 20–50 μl of the extracts were injected into the liquid chromatograph.

8-SO_4 in urine. For measurement of 8-SO_4 in urine, the urine was diluted 1:10 with distilled water, and 20–50 μl of diluted urine was injected into the liquid chromatograph.

Extraction of methoxy metabolites of fenoldopam from urine. For measurement of unconjugated methoxy metabolites, 1 ml urine, 100 μl of 0.05 *M* acetic acid (containing standards when preparing standard curve), and 100 μl internal standard (2.5 μg/ml IS-A and 11.7 μg/ml IS-C) were placed in a 100 × 17 mm polypropylene tubes. Ethyl acetate (5 ml) and saturated disodium phosphate solution (1.0 ml) were then added, and the tube was shaken for 10 min on a mechanical shaker. After phase separation by centrifugation, 4.5 ml of the ethyl acetate layer was transferred to another tube, containing 500 μl of 0.05 *M* hydrochloric acid. This tube was shaken for 10 min and then centrifuged. The ethyl acetate layer was aspirated and the tube was placed under nitrogen for 15 min to remove traces of ethyl acetate. Of the aqueous phase 10–50 μl was injected into the liquid chromatograph.

Hydrolysis of conjugated methoxy fenoldopam metabolites in urine. One ml urine was mixed with 100 μl of 0.05 *M* acetic acid (containing standards when preparing

standard curves), 1.0 ml acetate buffer (0.2 *M*, pH 4.7) and 100 μl diluted Glusulase enzyme solution. Samples were incubated for 16 h at 37°C, and the liberated methoxy compounds were extracted according to the method described for methoxy metabolites in urine.

Chromatography

A Model 110A HPLC pump (Beckman, Palo Alto, CA, U.S.A.) and an autoinjector (WISP model 710B, Waters Assoc., Milford, MA, U.S.A.) were used. All separations were carried out on an Ultrasphere 5 μm octadecyl silica column (25 cm × 4.6 mm I.D., Beckman). The electrochemical detectors consist of either a single glassy carbon electrode (for fenoldopam) or dual glassy carbon electrodes (for 8-SO_4 and methoxy metabolites), and an Ag/AgCl reference electrode. One (for single electrode) or two (dual electrode) LC-4B amperometric controllers (Bioanalytical Systems, West Lafayette, IN, U.S.A.) were employed. For fenoldopam assay, a potential of +0.65 V was maintained relative to the reference electrode. For the 8-SO_4 and methoxy metabolite assays, the dual glassy carbon electrodes were utilized in series, with a potential of +0.9 V on the upstream (W1) electrode and 0.0 V on the downstream (W2) electrode. Different mobile phases, A for fenoldopam, B for 8-SO_4 metabolite and C for methoxy metabolites were used at a flow-rate of 1.0 ml/min. The mobile phases were degassed by filtering through a 0.45-μm membrane filter (Type HA; Millipore, Bedford, MA, U.S.A.).

Quantitation

An appropriate standard curve was prepared for each set of samples. Peak height data were collected with a computer-automated laboratory system (Computer Inquiry Systems, Waldwick, NJ, U.S.A.) and peak height ratios, analyte *vs.* internal standard, were calculated. The equation for the linear regression line (fitted by least squares) of concentration *vs.* peak height ratio, was obtained for the standard curve. Analyte concentrations for samples were calculated from the following equation

$$\text{Concentration of analyte} = (c/d - a)/b$$

where: b = slope of the regression line; a = y-intercept of regression line; c = peak height for analyte; d = peak height for internal standard.

Validation procedures

Assays were validated in plasma and urine as follows: three pools (low, medium, and high concentrations) of the analyte were prepared by dissolving weighed amounts in known volumes of drug-free plasma or urine. On each of three days, at least five replicate samples from each pool were extracted and analyzed. Concentrations were determined by comparison with a standard curve, prepared freshly on the day of analysis. Validation studies were conducted for fenoldopam and 8-SO_4 in plasma; fenoldopam and methoxy metabolites in urine. Assays for 7-GLU, total conjugates, and methoxy conjugates were not validated because no authentic analytical standards were available. Since the 8-SO_4 assay in urine involves direct analysis of urine without prior sample treatment, no validation was necessary.

RESULTS AND DISCUSSION

Fenoldopam and its metabolites differ greatly in their stability and polarity, thus making it impractical to develop a single extraction procedure for all the compounds. The sulfate conjugates are very polar and stable. Methoxy metabolites are much less polar and have good stability. Fenoldopam has intermediate polarity and is less stable than any of its metabolites. Determination of the low levels of fenoldopam and its metabolites in the plasma and urine of man following therapeutic doses requires the sensitivity provided by ED. However, use of such detectors ruled out the possibility of developing a gradient elution system, due to the inherent problem of shifting baseline associated with these detectors. In view of the above difficulties, we developed separate extraction procedures and isocratic chromatographic systems for the routine analysis of these compounds in human plasma and urine.

Fenoldopam assay

Fenoldopam undergoes rapid decomposition when it is dissolved in water. However, it is stable for a period of at least 3 months at 4°C when dissolved in 0.05 *M* acetic acid. The addition of ascorbic acid to plasma and urine (0.5%, w/v) effectively stabilizes fenoldopam in these samples. Initial, short-term (1 month) stability studies in plasma and urine indicate that this compound is stable at −20°C when ascorbic acid is added to the media. Addition of ascorbic acid also increased the stability of fenoldopam in the final extract, because it was carried over to the final stage by the extraction procedure. The extracted samples were stable for at least 24 h at room temperature. Because addition of ascorbic acid lowered the pH of plasma and urine, it was necessary to add sodium hydroxide (for plasma) or phosphate buffer (to urine samples) in order to adjust the pH to 7.5, the optimum pH for the extraction of fenoldopam. An increase of the pH above pH 7.5 reduces the stability of ascorbic acid and fenoldopam.

Typical chromatograms of extracts of human plasma and urine, before and after oral administration of fenoldopam to healthy volunteers, are shown in Fig. 2. The retention times for fenoldopam and internal standard are 8.7 and 17 min, respectively. Due to variation among HPLC columns, slight manipulation of the methanol content of the mobile phase may be required to optimize the separation of fenoldopam from endogenous peaks. None of the known metabolites of fenoldopam or endogenous substances interfered either with fenoldopam or the internal standard. The lower limit of quantitation for fenoldopam was 50 pg/ml in plasma and 5 ng/ml in urine. Extraction recovery was determined by comparing detector response to standards injected directly on the column with the response to standards extracted from plasma and urine. The mean recovery for fenoldopam from plasma and urine was about 60%. Linear responses in fenoldopam *vs.* internal standard peak height ratios, with concentrations of fenoldopam from 50 pg–100 ng/ml plasma and 5 ng–5 μg/ml urine, were observed. Correlation coefficients exceeded 0.99 for all plasma and urine standard curves. The within-day and between-day precision of the plasma and urine methods are indicated by the coefficients of variation (C.V.) shown in Table I.

β-Glucuronidase should be used with caution in hydrolysis experiments, because the commercially available enzyme sources may contain some sulfatase activity.

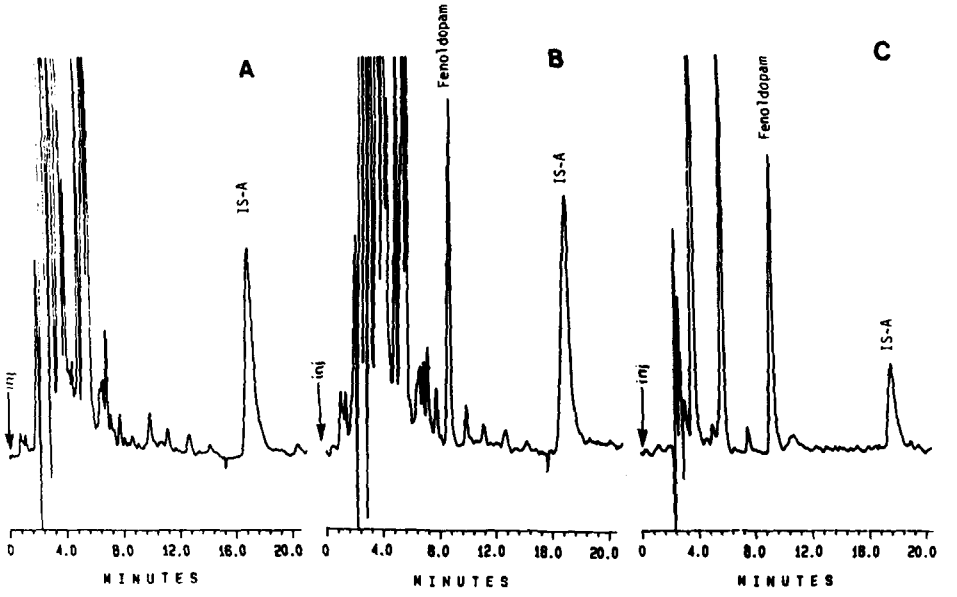


Fig. 2. Specific analysis of fenoldopam. Chromatograms of plasma extracts from a human subject before (A) and 2 h after (B) oral administration of 100 mg of fenoldopam and of a 0–24 h urine extract (C) following the dose. The concentration of fenoldopam was 2.6 ng/ml in plasma and 0.45 μg/ml in urine.

The sulfatase activity can be inhibited by adding 0.2 *M* phosphate buffer (pH 4.7) to the urine samples. Incubation of urine samples, spiked with high concentrations of 8-SO_4 metabolite (up to 300 μg/ml), with bovine liver β-glucuronidase (type B, Sigma, St. Louis, MO, U.S.A.) did not hydrolyze (less than 0.2% conversion after 16 h incubation) the sulfate metabolite in the presence of phosphate ions.

8-SO_4 metabolite assay

Initially, an HPLC method based on UV detection at 227 nm was developed

TABLE I

ACCURACY AND PRECISION DATA FOR FENOLDOPAM IN PLASMA AND URINE

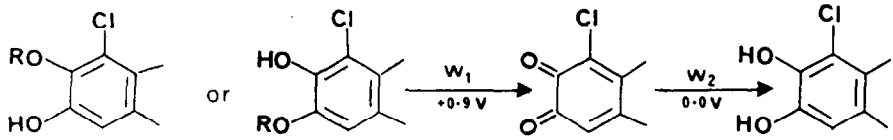
Type of sample	*Concentration*	*Assay conc.* Mean ± S.D.*	*Within-day C.V.***	*Between-day C.V.****
Plasma	0.25 ng/ml	0.27 ± 0.01	8.42	9.97
	2.5 ng/ml	2.45 ± 0.07	4.00	8.62
	50.0 ng/ml	52.08 ± 2.04	3.28	7.24
Urine	0.05 μg/ml	0.049 ± 0.005	6.93	2.08
	0.50 μg/ml	0.469 ± 0.014	2.18	4.49
	5.00 μg/ml	5.050 ± 0.190	4.14	0.88

* Mean value on day 1.

** (Day 1 C.V. + day 2 C.V. + day 3 C.V.)3.

*** $\frac{\text{Standard deviation of daily mean conc.}}{\text{Mean of individual daily mean conc.}} \times 100.$

for the determination of the 8-SO_4 metabolite in plasma. However, this method lacked the sensitivity (lower limit of detection is 100 ng/ml) and specificity needed for the routine analysis of this metabolite in plasma and urine following oral administration of fenoldopam. Preliminary information, obtained with dopamine sulfate, indicated that direct analysis of such conjugates might be feasible with dual-electrode ED[6]. Application of a high oxidative potential at the first electrode (W1 of a series configuration) cleaves the conjugate and, in the case of a catechol, produces the corresponding *o*-quinone. The quinone is subsequently reduced at the second electrode (W2) and the resulting current is measured. This novel technique was applied to the analysis of the 8-SO_4 conjugate and methoxy metabolites of fenoldopam. The dual glassy carbon electrodes in series first converted these metabolites to the quinone at 0.9 V (W1), and the reduction of the quinone was measured at the downstream electrode (W2) at a potential of 0.0 V (Scheme 1).



Scheme 1. Schematic representation of oxidation and reduction reactions occurring at thin-layer dual glassy carbon electrodes of an electrochemical detector in series configuration. R = CH_3 or SO_3^- or $C_6H_9O_6$.

Some initial success was achieved in detecting 8-SO_4 with a citrate–acetate buffer–10% methanol mobile phase. However, rapid passivation of W1 occurred, probably due to oxidation of impurities in the buffer. Use of 0.15 *M* monochloroacetate buffer (pH 3.2) in place of the citrate–acetate buffer prevented electrode passivation. Oxidation potentials greater than 0.9 V (W1) caused passivation of the electrode without an increase in sensitivity. Reduction potentials increasing to −0.20 V were evaluated. It was found that the more negative settings produced increased noise without gain in sensitivity. With the optimized potentials of 0.9 V (W1) and 0.0 V (W2) no loss in detector sensitivity was observed after six months of routine operation.

Typical dual-electrode chromatograms from extracts of human plasma, before and after administration of fenoldopam (100 mg) to a healthy volunteer, are shown in Fig. 3. The retention times for 8-SO_4 and IS-B are 14.8 and 26.9 min, respectively. Fig. 4 is the chromatogram obtained from injection of an aqueous standard solution of fenoldopam and its metabolites (except O-methyls) and IS-B. 8-SO_4 and IS-B are well separated from each other and also from the major and minor metabolites of fenoldopam. No endogenous peaks were found that interfered with either 8-SO_4 or IS-B. The lower limit of quantitation for 8-SO_4 in plasma was 2.5 ng/ml. Mean recovery for 8-SO_4 in plasma was 82%. Linear responses in 8-SO_4 *vs.* IS-B peak height ratio with concentrations of 8-SO_4 from 5.75–1725 ng/ml plasma were observed. The within-day and between-day precision of the method in plasma are indicated by the coefficient of variation (C.V.) shown in Table II.

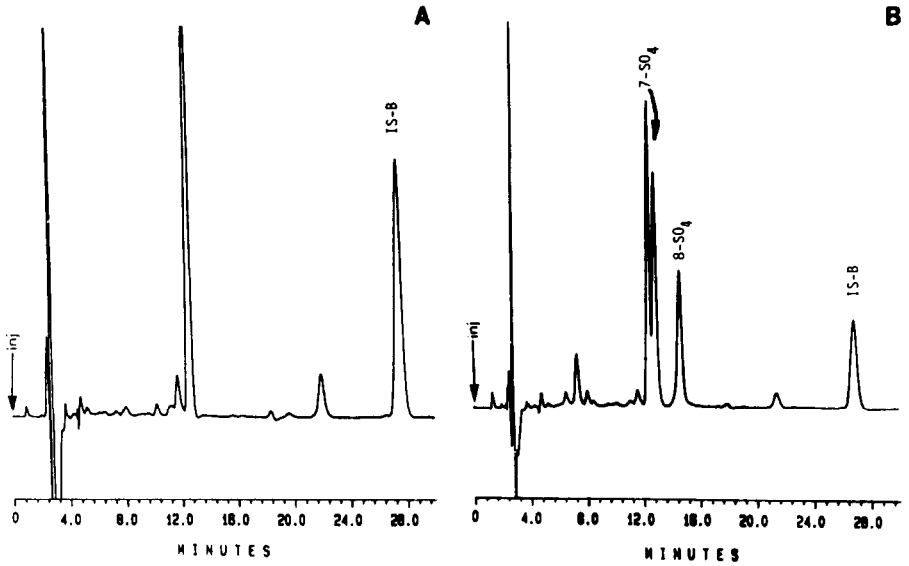


Fig. 3. Specific analysis of 8-SO_4. Chromatograms of plasma extracts from a human subject before (A) and 1.5 h after (B) oral administration of 100 mg of fenoldopam. The concentration of 8-SO_4 was 579 ng/ml.

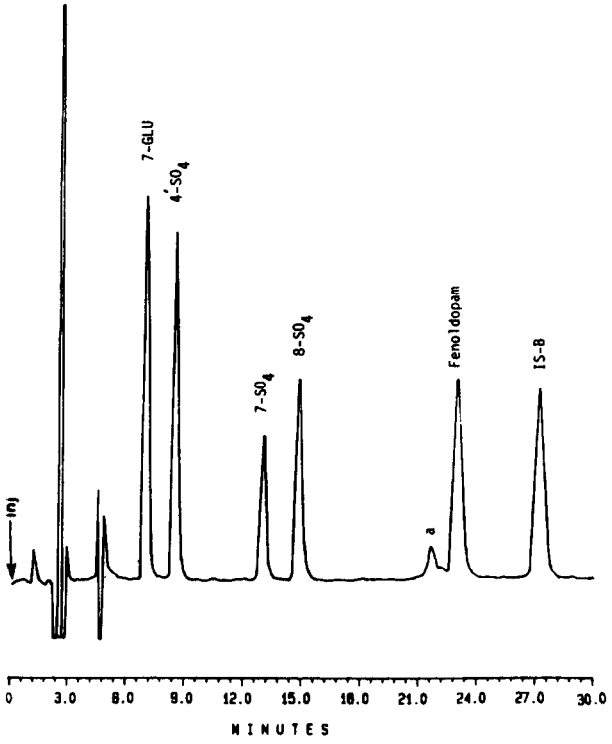


Fig. 4. Chromatogram of an aqueous standards solution of fenoldopam, its metabolite and the internal standard. (a) is the 8-sulfate isomer of IS-B, which is present as an impurity. Amounts of standards injected: 7-GLU (130 pmol), 8-SO_4 and 7-SO_4 (29 pmol each), fenoldopam (32 pmol) and IS-B (135 pmol).

TABLE II

ACCURACY AND PRECISION DATA FOR 8-SO_4, 7-MEO AND 8-MEO IN PLASMA AND URINE

Analyte	*Type of sample*	*Conc. (ng/ml)*	*Assay conc. Mean ± S.D.*	*Within-day C.V.*	*Between-day C.V.*
8-SO_4	Plasma	11.5	11.1 ± 0.3	6.68	2.03
		230.0	227.1 ± 12.0	4.31	0.93
		1380.0	1366.0 ± 32.0	2.80	3.48
7-MEO	Urine	23.0	23.6 ± 0.6	3.81	1.12
		230.0	242.8 ± 5.1	1.59	0.50
		2300.0	2286.0 ± 23.0	2.28	2.26
8-MEO	Urine	23.0	24.5 ± 1.4	4.47	4.09
		230.0	241.4 ± 5.6	1.97	2.55
		2300.0	2307.0 ± 23.0	1.82	2.60

In urine, 8-SO_4 was measured directly by injecting the diluted urine samples into the column and comparing the response with a standard solution of 8-SO_4. Fig. 5 shows chromatograms obtained from injection of diluted urines, obtained before and after administration of fenoldopam to a healthy volunteer. Analysis of numerous control urines has shown no interference from endogenous compounds.

The plasma and urinary levels of 7-SO_4 can also be quantitated employing the above methods. Because the quantity of analytical standard available was insufficient, the assay was not validated.

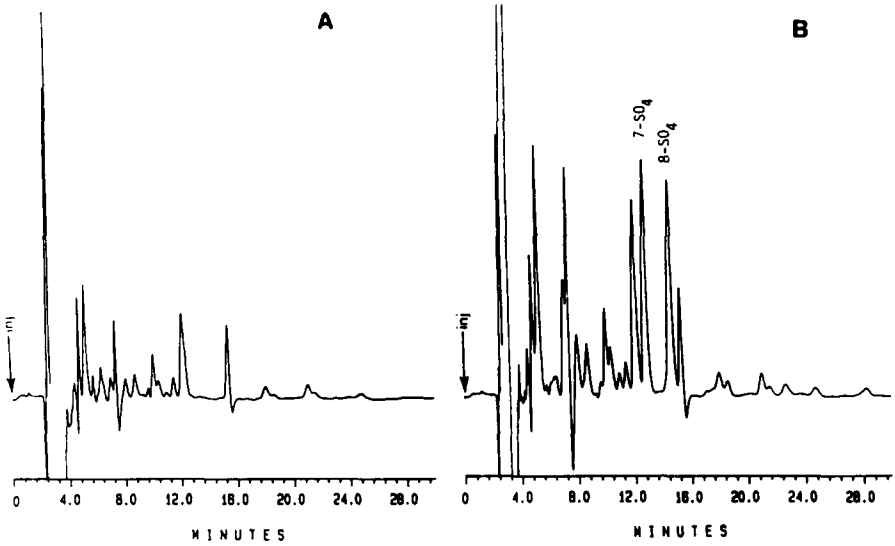


Fig. 5. Specific analysis of 8-SO_4. Chromatograms of urine from a human subject before (A) and 0–24 h (B) after ingestion of 100 mg fenoldopam. The concentration of 8-SO_4 was 12.33 μg/ml.

Methoxy metabolites assay

Since the methoxy metabolites of fenoldopam exist in urine primarily as conjugates, analysis was conducted only after enzymatic hydrolysis with Glusulase. Because these metabolites are relatively non-polar, a stronger mobile phase (C) was employed to obtain practical retention times. As described under the assay of 8-SO_4, dual-electrode ED provided direct analysis of these metabolites. Two internal standards (IS-A and IS-C) were used at concentrations sufficient to span the wide range of concentrations (5.75–5750 ng/ml) of methoxy metabolites in the urine. For concentrations up to 5000 ng/ml, IS-C was used to calculate peak height ratios, and above this concentration, IS-A was used.

Fig. 6 shows typical dual-electrode chromatograms of extracts from hydrolyzed urines, obtained before and after oral administration of fenoldopam to a healthy volunteer. The retention times for IS-A, 8-MEO, 7-MEO and IS-C are 7.4, 9.11, 12.4 and 17.4 min, respectively. No endogenous peaks were found that interfered with the methoxy metabolites of fenoldopam or with the internal standards. The lower limit of quantitation for 7-MEO and 8-MEO were 2.5 ng/ml. Mean recoveries for 7-MEO and 8-MEO in urine were 94 and 93%, respectively. Linear responses in 7-MEO or 8-MEO *vs.* IS-C peak height ratios, with concentrations of 7-MEO or 8-MEO from 5.75 to 5750 ng/ml urine, were observed. The within-day and between-day precision of the urine method are indicated by coefficients of variation (C.V.) shown in Table II.

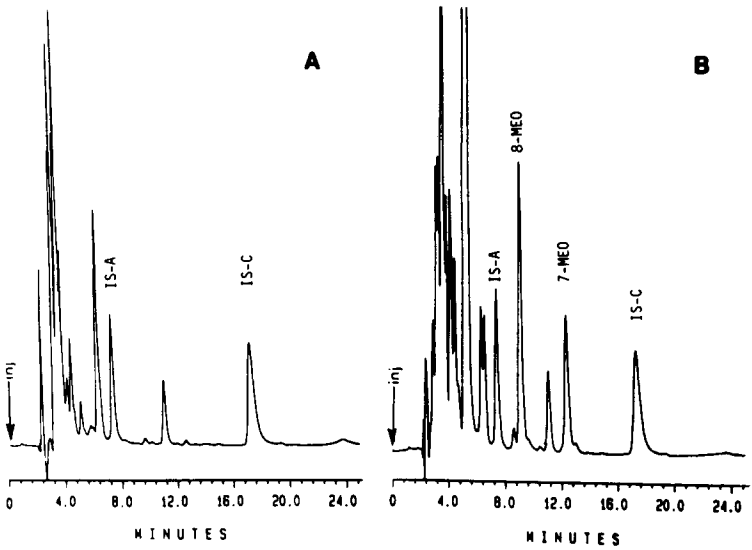


Fig. 6. Specific analysis of methoxy metabolites of fenoldopam. Chromatograms of hydrolysed urine extracts from a human subject before (A) and 0–24 h (B) after ingestion of 100 mg fenoldopam. The concentrations of 7-MEO and 8-MEO were 1578 and 2180 ng/ml, respectively.

These assay methods were routinely used for the analysis of fenoldopam and its metabolites in plasma and urine. Over 2000 plasma and urine samples from various clinical studies were analyzed by these methods. In conclusion, we have described very specific and sensitive HPLC methods with ED for fenoldopam and its metab-

olites in plasma and urine, which are suitable for pharmacokinetic and disposition studies of this compound. In addition, a novel method is described for the analysis of catechol sulfates and O-methyl catechols based on dual-electrode HPLC–ED. Preliminary data indicate that this method promises to have general utility in the analysis of structurally related catechol metabolites, in which one phenolic group has been conjugated with either a sulfate, glucuronic acid, or methyl group.

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