Quantitation of Pentisomide and its Metabolite by High-Pressure Liquid Chromatography in Human Serum

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Abstract

A rapid, inexpensive, and accurate high-performance liquid chromatographic method for the simultaneous determination of pentisomide and its major mono-*N*-dealkylated metabolite has been developed. After a simple and inexpensive solvent extraction procedure, the unchanged drug, its metabolite, and the internal standard are separated using a C_{18} reversed-phase column with a 5-µm particle size. The eluent is monitored with ultraviolet detection at 260 nm. Endogenous substances or a variety of drugs do not interfere with the assay. The mean recoveries of pentisomide and its metabolite are 92.6% and 92.2%, respectively. The limit of detection of the assay is 28 ng/mL for both drugs. Serum levels of pentisomide and its metabolite in patients on oral therapy for supraventricular tachycardia are reported.

Introduction

Pentisomide, 2-[2-diisopropylamino)-ethyl]-4-methyl-2-(2-pyridyl)-pentanamide, a derivative of disopyramide (1), is a new Class I antiarrhythmic drug currently under investigation for ventricular (2–4) and supraventricular (5–8) arrhythmias. Experimental studies indicate that its main action is the inhibition of the fast sodium channel (9–11), whereas its calcium blocking effects are probably not of clinical relevance (12).

Although the efficacy of antiarrhythmic drugs often does not correlate directly with serum level, the determination of serum levels is recommended where drug therapy is ineffective in order to differentiate failure of therapy from suboptimal dosing. This procedure is especially important for elderly patients with decreased creatinine clearance (13).

The aim of the present study was to develop a simple but accurate high-performance liquid chromatographic (HPLC) method to determine pentisomide and its mono-*N*-dealkylated metabolite in human serum.

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Experimental

Chemicals and reagents

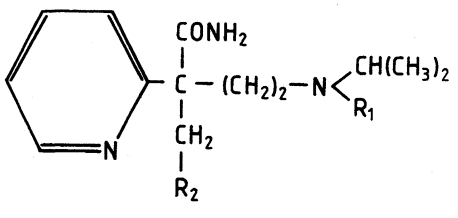
Pentisomide (CM7857), its metabolite mono-*N*-dealkyl-pentisomide (CM40534), and the internal standard (CM7973) were provided by Midy-Sanofi (Montpellier, France). All chemical structures are shown in Figure 1. Acetonitrile, hydrochloric acid, triethylamine, dichloromethane, and sodium hydroxide were obtained from Merck (Darmstadt, Germany), and 1-octanesulfonic acid (PIC B8 low UV reagent) and dibutylamine phosphate (PIC D4 reagent) were obtained from Waters (Eschborn, Germany).

Apparatus

The HPLC system used consisted of a Model 721 system controller, a Model 510 HPLC pump, a WISP 710B injector block, a Lambda-Max Model 481 variable-wavelength detector, and a Data Module Model 730 (Waters Associates). The absorbance spectrum of pentisomide and the metabolite were established using a spectrophotometer (Perkin-Elmer 15 UV/VIS; Überlingen, Germany).

Chromatographic conditions

The analysis was performed using a Shandon Hypersil-ODS reversed-phase column (250 × 4.6-mm i.d., 5-µm particle size)



R_1	R_2	
$CH(CH_3)_2$	$CH(CH_3)_2$	Pentisomide
H	$CH(CH_3)_2$	Metabolite
$CH(CH_3)_2$	CH_3	Internal Standard

Figure 1. Chemical structures of pentisomide, its metabolite, and the internal standard.

(Grom, Herrenberg, Germany) at room temperature (20°C). The mobile phase consisted of 74% v/v eluent A (962 mL water, 25 mL PIC B8 low UV reagent, 1 mL triethylamine, 12 mL PIC D4 reagent) and 26% v/v eluent B (acetonitrile). The flow rate was 1.0 mL/min. The column effluent was monitored at 260 nm using a detector range of 0.02 AUFS and a chart speed of 0.5 cm/min. The injected volume was 200 µL.

Standards

Three stock standard solutions were prepared; each contained 20 µg/mL pentisomide, its metabolite, or an internal standard in 0.01M hydrochloric acid. The solutions were stable for at least 4 months if stored at 4°C. A standard working solution was obtained by combining aliquots of the stock solutions and diluting them with hydrochloric acid to concentrations of 2.0 µg/mL for pentisomide and its metabolite and 3.0 µg/mL for the internal standard.

Sample collections

Serum was obtained by centrifugation at 900 × *g* for 10 min. Twenty-two serum samples from normal volunteers (12 male, 10 female) were pooled (normal pool). Serum levels of pentisomide and its metabolite were determined in 12 patients on the fifth day of oral treatment with pentisomide for control of supraventricular tachycardia. Patients received 900 mg pentisomide per day if their bodyweight was 80 kg or less; they received 1200 mg per day if their bodyweight was above 80 kg. Serum samples from the 12 patients were taken before and 3 h after oral intake; the results were 900 mg and 1200 mg pentisomide, respectively.

Extraction procedure

Dichloromethane (2 mL), serum (1.0 mL), 0.1M sodium hydroxide (100 µL), and the stock solution of the internal standard (100 µL) were introduced into a 10-mL glass centrifuge tube (Kästner; Tübingen, Germany). The tube was closed with a stopper (Sarstedt; Nürnbrecht, Germany), shaken for 1 min, and centrifuged at 3600 × *g* for 10 min. The serum phase was subsequently discarded, and 1.0 mL of the dichloromethane phase was transferred to a clean glass tube and evaporated to dryness at room temperature (20°C) under a stream of nitrogen. The residue was redissolved in 500 µL of 0.01M hydrochloric acid.

Linearity test

The linearity of the chromatographic procedure was tested for pentisomide, its metabolite, and the internal standard by analyzing eight standard solutions with concentrations of 10.0, 5.0, 2.5, 1.25, 0.63, 0.31, 0.16 and 0.08 µg/mL for each of the components. The solutions were obtained by diluting the standard solutions containing pentisomide, its metabolite, and the internal standard. At each concentration step, three determinations were performed. Each standard (100 µL) was introduced into a glass centrifuge tube, and 1.0 mL of the normal pool serum was added. The spiked serum samples were extracted and subjected to chromatographic analysis. Three determinations were performed at each concentration.

Table I. Recovery of Pentisomide and Metabolite from Plasma

Concentration (µg/mL)	Recovery (%)	
	Pentisomide (n = 10)	Metabolite (n = 10)
0.5	89.8	87.0
2	91.1	93.8
5	93.5	92.5
10	95.9	94.0
Mean ± SD	92.6 ± 2.7	92.2 ± 2.8

Recovery rates

The recovery rates were determined by comparing the peak areas of pentisomide, its metabolite, and the internal standard, which were obtained by analyzing a spiked serum sample (100 µL of each stock solution standard plus 1.0 mL of the normal pool), with the peak areas obtained by direct injection of the standard working solution (2.0 µg/mL pentisomide, 2.0 µg/mL metabolite, and 3.0 µg/mL of the internal standard). This procedure was repeated with different drug concentrations (see Table I).

Quantitation

Prior to injection of each patient probe, the standard working solution of pentisomide, its metabolite (2.0 µg/mL each), and the internal standard (3.0 µg/mL) were injected. Because there was a linear relationship between the peak area and the concentration of pentisomide and its metabolite, concentrations of pentisomide or its metabolite in the patient probe were calculated using the following equation:

$$C_P = \frac{A_P}{A_S} \times \frac{100}{R_{IS}} \times C_S \qquad \text{Eq 1}$$

where A_P is the peak area of pentisomide or the metabolite in the patient probe; A_S is the peak area of pentisomide or the metabolite in the standard working solution; C_P is the concentration of pentisomide or the metabolite in the patient probe; C_S is the concentration of pentisomide or the metabolite in the standard solution; and R_{IS} is the recovery rate of the internal standard. Both concentrations are given in micrograms per milliliter.

Table II. Drugs Tested for Interference with the Determination of Pentisomide or its Metabolite by HPLC

Acebutolol	Digoxin	Paracetamol
Acenocoumarol	Diazepam	Pindolol
Acetylsalicylatacid	Diltiazem	Propafenon
Allopurinol	Disopyramide	Quinidine
Amiloride	Furosemide	Ranitidine
Amiodarone	Flecainide	Sotalol
Baclophen	Heparine	Spironolactone
Benceracid	Isosorbitmononitrat	Sulfinpyrazone
Betaxolol	Metoprolol	Thiazide
Bezafibrate	Nifedipine	Verapamil
Digitoxin	Nitrendipine	Xipamid

Because the recovery of pentisomide and the metabolite in the patient probe might change in various extractions, it was calculated by dividing the peak area of the internal standard of the patient probe with the peak area of the internal standard in the standard working solution (Equation 2). The recovery rates of all three substances were within 5%. The concentration of pentisomide and the metabolite in the patient probe was determined (micrograms per milliliter) by taking into account the concentrations of the two substances in the standard working solution (C_S in Equation 1).

$$\frac{A_{PI} \times 100}{A_{SI}} = \text{recovery rate (\%)} \qquad \text{Eq 2}$$

A_{PI} is the peak area of the internal standard in the patient probe, and A_{SI} is the peak area of the internal standard in the standard working solution.

Results

For the standards, as well as for the entire procedure, there was a linear relationship between concentration and peak area over the total range tested. The lower limit of quantitation of all three substances was 0.028 g/mL. The recovery rates of 10 determinations (mean plus or minus standard deviation) obtained with four different drug concentrations (see Table I) were 92.6 ± 2.7% for pentisomide, 92.2 ± 2.8% for the metabolite, and 95.3 ± 2.9% for the internal standard. These recovery rates were consistent across the high and low concentrations (see Table I). Serum levels of one serum sample determined repeatedly over the course of 1 year were stable (storage at –20°C). We did not observe any interference in the chromatographic procedure by other drugs as listed in Table II.

The precision of the assay was assessed by replicate analyses of spiked plasma samples containing pentisomide and its metabolite at concentrations of 1, 2, and 5 µg/mL. The intra-assay and inter-assay coefficients of variation are presented in Table III. The within-day and between-day variation at each concentration for both drugs was less than 7%.

The serum levels of pentisomide and the metabolite before and 3 h after oral intake on the fifth day of treatment with pentisomide are presented in Table IV. Serum levels of pentisomide were not different for patients treated successfully with pentisomide and patients in whom the tachycardia was still inducible in an electrophysiologic study. Patients with low drug concentrations did not take a different concomitant medication than patients with higher drug levels.

A typical chromatogram obtained from the analysis of a serum sample of a patient receiving pentisomide (900 mg/day) is shown in Figure 2.

Table III. Precision Data for the Determination of Pentisomide and its Metabolite

Concentration (µg/mL)	Within-day (n = 10)		Between-day (n = 10)	
	Pentisomide (%)	Metabolite (%)	Pentisomide (%)	Metabolite (%)
1	3.8	2.9	6.2	5.8
2	2.4	1.8	5.5	6.0
5	2.0	1.6	3.9	5.1

Table IV. Serum Levels of Pentisomide and its Metabolite Before and After Oral Intake of 900 mg or 1200 mg Pentisomide

Dosage (mg)	Serum level (g/mL)			
	Metabolite		Pentisomide	
	Before	3 h	Before	3 h
900	0.49	0.76	1.17	2.43
900	0.73	0.91	2.80	4.00
900	0.66	1.21	2.42	3.87
900	0.47	0.86	1.59	3.36
900	1.02	0.76	2.84	3.24
900	0.54	0.78	2.54	3.92
Mean ± SD	0.65 ± 0.21	0.88 ± 0.17	2.23 ± 0.69	3.47 ± 0.60
1200	0.62	0.75	2.23	4.49
1200	0.55	0.88	2.71	4.56
1200	0.63	1.07	1.85	3.71
1200	0.35	0.69	1.98	3.61
1200	0.99	1.02	3.24	2.81
1200	1.24	1.53	3.85	4.84
Mean ± SD	0.73 ± 0.32	0.99 ± 0.30	2.64 ± 0.78	4.00 ± 0.76

Discussion

Three HPLC methods that use ultraviolet detection (14–16) and one gas chromatographic (GC) method that uses thermionic detection (17) have been described for measurement of pentisomide in human serum.

The main disadvantage of the GC methods is that additional steps, such as separate assays or derivatization procedures, are required to determine metabolite concentrations. HPLC techniques allow simultaneous assay of the drug and its major metabolite in the same sample with use of a common internal standard. Reliable determinations of the metabolite are important because drug metabolites may add or alter the effects of the parent compound by exerting similar actions, competing with the parent compound, or mediating drug toxicity (18).

Necciari and co-workers (17) reported recovery rates of pentisomide ranging from 59.3% to 67.5%. Houin and co-workers (14) also demonstrated a relatively moderate recovery of 60% and 69% for the metabolite

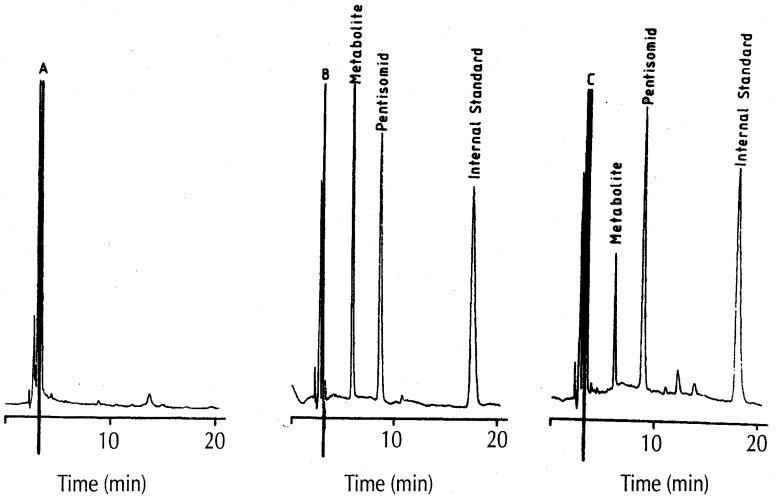


Figure 2. Chromatograms of a blank from the normal pool (A), a standard working solution with 2 µg/mL pentisomide (B), and the serum of a patient receiving 900 mg pentisomide (C) (concentration found was 3.24 µg/mL).

and pentisomide, respectively, probably due to the complicated liquid–liquid extraction procedure, which was used by both authors (14,17). This procedure requires a back extraction into an acidic buffer and contains altogether three phase separations consuming nearly 60 min. Walker and co-workers (15) achieved satisfactory recovery for pentisomide (95% ± 1.9%) but needed a high sensitivity assay in addition to a direct-extraction procedure in order to improve the recovery rate of the metabolite from 74% ± 1.6% to 79% ± 2.5%. In addition, only three determinations for each substance were performed to evaluate the respective recovery rates. Furthermore, the high sensitivity assay was necessary to detect drug levels below 0.5 mg/L. Because it is difficult to predict drug levels after antiarrhythmic therapy (19), two extraction procedures might be necessary to obtain samples ready for HPLC injection, which would result in a waste of specimens. The HPLC method described by Plomp and Buijs (16) involves extraction of samples via activated Bond Elut C_8 disposable columns that require repeated washing procedures with methanol, water, and acetonitrile. In addition, the collected eluates have to be evaporated to dryness under a nitrogen stream and reconstituted in the mobile phase used in that assay. This is a potent extraction procedure with recoveries of 91.1% for pentisomide, 93.8% for the metabolite, and 90% for the internal standard, but such equipment is not available in all clinical laboratories. Because these extraction columns may be reused at least five times, this method is far more expansive than our extraction procedure, which is supposed to be used for a large number of samples in clinical practice.

The mean recovery rates in our extraction procedure were 92.2% ± 2.8% for the metabolite and 92.6% ± 2.7% for pentisomide. All necessary steps for extraction required approximately 15 min. With the dichloromethane–serum ratio used, we did not see any emulsification. Ultraviolet detection was performed at 260 nm, which was the determined absorbance peak for pentisomide and its metabolite. Wavelengths of 254 nm (14) and 258 nm (17) were used in other reports.

The metabolite, pentisomide, and the internal standard were eluted with retention times of 6.3, 8.5, and 16.9 min, respectively, whereas the retention times of pentisomide and its metabolite reported by the other methods occurred within 3 min; thus there were possible peak interferences. The specificity of the method is excellent, as demonstrated by the lack of interference from endogenous material. No interference was noted after the addition of 29 drugs that may be administered with pentisomide. Even disopyramide and the beta-blocking agents acebutolol and metoprolol did not interfere with pentisomide, contrary to other reports (15–17). Serum samples can be stored at –20°C for at least a year without deterioration of the drug.

This method is accurate, rapid, and inexpensive. It is suitable for monitoring therapeutic and toxic serum levels of pentisomide and its metabolite.

We determined pentisomide and metabolite serum levels in patients treated with pentisomide for supraventricular tachycardia. The determined serum levels showed a high interindividuel variability. This variability was independent of concomitant drug medication or impaired renal function; efficient control of supraventricular tachycardia and side effects were not dependent on pentisomide or its metabolite serum level in this small patient group. Plasma levels of pentisomide and its metabolite are in good agreement with previous reported plasma levels (16). Most patients treated with 1200 mg pentisomide had drug levels above those of patients treated with the minor dosage (900 mg), though bodyweight was the factor used to determine treatment dosage.

Conclusion

This newly developed, sensitive, and selective HPLC method is suitable for simple, rapid, and accurate monitoring of serum levels of pentisomide and its metabolite in clinical practice. Serum levels were determined in patients after oral treatment with pentisomide for control of supraventricular tachycardia.

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