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High-performance liquid chromatographic method for the simultaneous determination of pentisomide and its major metabolite N-desisopropylpentisomide in plasma, urine and tissues using solid-phase extraction

Theo A. Plomp and Maurice J. H. Buijs

Netherlands Institute for Drugs and Doping Research, University of Utrecht, Vondellaan 14, 3521 GE Utrecht (Netherlands)

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ABSTRACT

A rapid, sensitive and selective high-performance liquid chromatographic method for the simultaneous determination of pentisomide and its major metabolite desisopropylpentisomide in plasma, urine and tissues has been developed. The method for plasma samples, urine samples and tissue samples, after homogenizing with 50% ethanol, involves extraction of samples via activated Bond-Elut C_8 disposable columns with methanol at pH 10 after addition of internal standard, and initially on column washings of samples at pH 10 with water and acetonitrile. The obtained methanolic extract is evaporated to dryness under nitrogen at 25°C; the sample residue is then reconstituted in mobile phase and an aliquot of this solution is injected into the liquid chromatograph. Separation is performed using a Nova-Pak C_{18} 4 μm particle size column operating in combination with radial compression separation unit and a methanol–water–di-*sec*.-butylamine–phosphoric acid (40:60:0.5:0.2, v/v) pH 3.5 mobile phase with ultraviolet detection at 258 nm. Endogenous substances or a variety of drugs concomitantly used in pentisomide therapy, with the exception of disopyramide, do not interfere with the assay. The mean recovery of pentisomide and desisopropylpentisomide from plasma and urine and from tissues is more than 91 and 86%, respectively. The limit of detection of the assay is 10 ng/ml for both drugs. The intra- and inter-day coefficient of variation for replicate analyses of spiked plasma samples is less than 7 and 8%, respectively. Mean steady-state plasma levels of pentisomide and desisopropylpentisomide in patients on chronic oral therapy are reported.

INTRODUCTION

Pentisomide, *dl*-2-[2-(diisopropylamino)ethyl]-4-methyl-2-(2-pyridyl)pentamide (other generic names penticainide, propisomide, CM 7857 and ME 3202; Fig. 1), is a new synthetic anti-arrhythmic agent, structurally related to disopyramide [1], which has demonstrated to be effective in experimental and clinical arrhythmias [1–5]. Based on *in vitro* studies, pentisomide has been classified as a class I anti-arrhythmic agent. It has proved to have class IB properties, such as shortening of action potential duration, as well as class IC properties, such as slow recovery kinetics of rate-dependent block [6–9].

The first clinical studies have indicated that pentisomide seems to be a well-tolerated and effective drug of potential value for management of supraventricular and ventricular arrhythmias [3–5]. Single intravenous and oral dose pharmacokinetics of pentisomide in healthy volunteers and in patients with cardiac arrhythmias have been reported by several investigators [3,10,11]. A mean plasma elimination half-life for pentiso-

Correspondence to: Dr. T. A. Plomp, Laboratory of Centrafarm Services, Nieuwe Donk 9, 4879 AC Etten-Leur, Netherlands.

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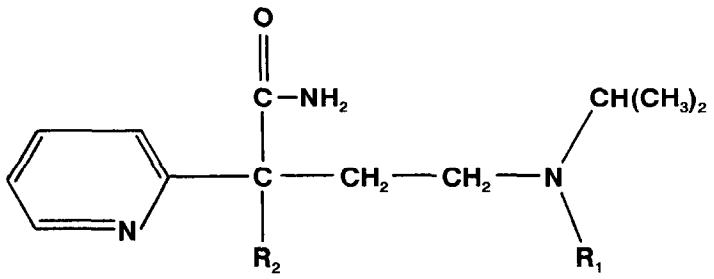

COMPOUND	R_1	R_2
Pentisomide	$CH(CH_3)_2$	CH_2-$CH(CH_3)_2$
Desisopropylpentisomide	H	CH_2-$CH(CH_3)_2$
Internal Standard	$CH(CH_3)_2$	$(CH_2)_4$-CH_3

Fig. 1. Chemical structures of pentisomide, desisopropylpentisomide and the internal standard used in this assay.

mide varying from 11 to 15 h was observed in these studies [3,10,11].

The disposition and metabolism of pentisomide have been investigated in various animal species and man by Davi *et al.* [12] using a single oral administration of ^{14}C-labelled pentisomide. They found that the major route of metabolism in man is N-dealkylation of the di-isopropyl moiety to give desisopropylpentisomide (CM 40534, Fig. 1). After an oral dose, about 95% of the administered dose was excreted in urine, of which about 60% was unchanged drug, 30% was in the form of the main metabolite desisopropylpentisomide and 7% was in the form of different other metabolites.

A gas chromatographic method for the determination of pentisomide in plasma and urine using nitrogen-selective detection was reported by Necciari *et al.* [13]. The method for plasma samples consisted of an extraction of pentisomide after addition of sodium carbonate buffer pH 12 with methylene chloride and back-extraction of the drug from the organic phase into aqueous acid medium followed by re-extraction of the drug from the aqueous phase after addition of strong alkali with methylene chloride. The methylene chloride layer was transferred and evaporated to dryness under a stream of nitrogen. The residue was redissolved in methanol and an aliquot of this solution was injected onto a 3% OV17 column using a thermionic detector.

The method for urine samples included direct extraction with methylene chloride from strong alkaline medium without further clean-up. After centrifugation and evaporation of the organic layer to dryness, the obtained residue was processed as described for plasma samples. This method is specific and sensitive with a detection limit for pentisomide in plasma and urine of 25 and 50 ng/ml, respectively. However, its extraction procedure is relatively complex and time-consuming and the recovery from plasma was moderate and varied from 59 to 68%. A high-performance liquid chromatographic (HPLC) method for the simultaneous determination of pentisomide and its dealkylated metabolite in plasma and urine has been reported, using a reversed-phase C_{18} column and UV detection at 254 nm [14]. The extraction procedure used for the drug and its metabolite was similar to the above-described method of Necciari *et al.* [13]. The detection limit of the HPLC method was about 25 ng/ml for both compounds, and the mean overall analytical recoveries of the parent

drug and the metabolite from plasma were 60% and 69%, respectively. The method showed an adequate sensitivity, selectivity and reproducibility for both compounds, however, a multiple extraction was required in the work-up of the samples and the recovery from plasma was moderate.

A simple HPLC method for the measurement of pentisomide and desalkylpentisomide in plasma and urine, using a normal-phase silica column and UV detection at 260 nm, has been developed by Walker *et al.* [15]. The method is based on a direct single extraction of a small sample volume (200 μl) at strongly basic pH with a mixture of isoamyl alcohol and methyl *tert.*-butyl ether (10:90, v/v) and after centrifugation direct analysis of the extract.

For the assay of low levels of the drugs (10–500 ng/ml), larger sample volumes and prior concentration of the extract by evaporation of the organic phase to dryness with air and dissolution of the residue in small volume of eluent have been used. The advantages of this method are a simple extraction procedure, a high specificity and sensitivity with a detection limit for both compounds of 10 ng/ml and an adequate extraction efficiency of 95% for pentisomide. The major disadvantages are the relatively moderate recovery of 74% for the metabolite and the lack of applicability of this method for tissue analysis. In this report, a rapid, sensitive and specific HPLC method for the determination of pentisomide and desisopropylpentisomide concentrations in plasma, urine and tissues is described. It includes a simple bonded-phase extraction column procedure followed by analysis on a reversed-phase C_{18} column with ultraviolet detection. This method was used to measure the parent drug and its metabolite in plasma samples obtained from patients on pentisomide treatment and is acceptable for *post mortem* tissue analysis.

EXPERIMENTAL

Chemicals and reagents

Pentisomide (CM 7857), desisopropylpentisomide dihydrochloride (CM 40534) and the internal standard [2-(2-diisopropylaminoethyl)-2-(2-pyridyl)heptamide, CM 7973] (Fig. 1) were all provided by Sanofi Research Center (Montpellier, France). HPLC-grade methanol and HPLC-grade acetonitrile were purchased from Baker (Deventer, Netherlands). Di-*sec.*-butylamine for synthesis was purchased from Merck Schuchardt (Munich, Germany). Anhydrous sodium carbonate and orthophosphoric acid (85%) were of analytical grade and were obtained from Merck (Darmstadt, Germany). An aqueous solution of 0.2 *M* sodium carbonate, pH *ca.* 12.3, was prepared by dissolving 21.2 *g* of anhydrous sodium carbonate in 1 litre of double-distilled water. Bond-Elut 200-mg C_8 disposable extraction columns were obtained from Analytichem International (Lawndale, CA, USA).

Instruments

The HPLC system consisted of a Model 6000A solvent delivery pump, a Model U6K injector, a Guard-Pak pre-column module, a radial compression separation unit and a Model 481 Lambda-Max variable-wavelength detector, all from Waters (Milford, MA, USA). The radial compression separation unit consisted of a 10 cm × 8 mm I.D. Nova-Pak C_{18} (4 μm, RP-18) cartridge and a Model RCM-100 module for compressing the cartridges. The separation unit was preceded by the Guard-Pak pre-column module equipped with a μBondapak C_{18} disposable (8 mm × 8 mm I.D.) pre-column insert packed with 4-μm Nova-Pak C_{18} particles. The output of the detector was displayed on a Varian Model A-25 recorder. The output signal was also fed to a Laboratory Computing Integrator Model LCI-100 (Perkin-Elmer) for integration of peak areas. An all-glass filter apparatus with appropriate 0.45-μm filters (Solvent Clarification Kit, Waters Assoc.) was employed to filter methanol and bi-distilled water before use in the mobile phase.

The sample preparation system consisted of a Baker-10 SPE vacuum manifold system (J. T. Baker, Phillipsburg, PA, USA) that can simultaneously process up to ten samples. The disposable columns used for the sample extraction procedure were Bond-Elut columns containing 200 mg of octyl (C_8) reversed-phase packing material

and having a column volume of 3 ml. The extraction columns were activated prior to use by attachment to the Baker-10 SPE vacuum system and double washing with 3 ml of methanol followed by double washing with 3 ml of distilled water. Each of the washings was drained by applying a vacuum of 34 kPa using a water pump attached to the Baker-10 SPE system. Other equipment included a Potter homogenizer (Type E6, Heidolph Elektro, Kelheim, Germany), a vortex-type mixer and a high-speed centrifuge.

Standard solutions

Stock standard solutions containing 1000 μg/ml pentisomide base, desisopropylpentisomide base and internal standard were made in 0.1 *M* hydrochloric acid. Stock mixed solutions containing, respectively 100 and 10 μg/ml pentisomide and desisopropylpentisomide in 0.02 *M* hydrochloric acid were made from the stock standard solutions of the drugs. Internal standard solutions containing 100 and 5 μg/ml in 0.02 *M* hydrochloric acid were prepared by dilution of the stock internal standard solution with bidistilled water. Standard solutions in plasma or urine containing pentisomide and desisopropylpentisomide at concentrations of 0.10, 0.25, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00 and 10.00 μg/ml were made by appropriate dilution of the stock mixed standard solutions with plasma and urine respectively. In addition, urine standards containing 20 and 50 μg/ml of pentisomide and desisopropylpentisomide, respectively, were prepared.

Calibration standards in mobile phase containing 5 μg/ml internal standard and 0.1–10.0 μg/ml pentisomide and desisopropylpentisomide were made by diluting the stock mixed standard solutions with mobile phase. The stock solutions and calibration standards in mobile phase were stored at 4°C and were stable for at least three months if stored at this temperature. The plasma and urine standards were stable for six weeks if stored at −20°C in disposable polypropylene-capped glass centrifuge tubes (100 mm × 16 mm).

Tissue standards containing 1–50 μg/g of both drugs were prepared by homogenizing 100 mg of finely minced tissue for 1 min with a mixture of 1.00 ml of ethanol, 10–500 μl of stock mixed standard solution of 100 μg/ml and 990–500 μl of 0.1 *M* hydrochloric acid in a glass tube with a homogenizer.

Chromatographic conditions

All chromatography was done at ambient temperature. The mobile phase was a mixture of methanol–water–di-*sec*.-butylamine (40:60:0.5, v/v) brought up to pH 3.5 with phosphoric acid. The flow-rate was 1.0 ml/min, maintained by a pressure of approximately 66 bar. The column was conditioned with mobile phase for 48 h before use. The column effluent was monitored at 258 nm, the absorbance maximum of pentisomide, desisopropylpentisomide and internal standard in methanol, using a detector range of 0.005 a.u.f.s. and a chart speed of 0.2 cm/min.

Extraction procedure for plasma, urine and tissues

Plasma and urine. To the top of activated disposable C_8 extraction columns 1 ml of 0.2 *M* sodium carbonate solution, 1.00 ml of plasma or diluted urine (five-fold with 0.9% sodium chloride solution) sample and 1.00 ml of internal standard solution in 0.02 *M* hydrochloric acid (5 μg/ml) were added successively. After waiting for 1 min, the solutions were slowly eluted through the columns by application of a vacuum of 7–10 kPa. The columns were subsequently washed twice with 3 ml of water and twice with 1 ml of acetonitrile under vacuum of 34 kPa. The vacuum was then released and sample collection tubes were placed in the manifold system under each column. Methanol (0.50 ml) was added to the columns, which were then allowed to stand for 1 min prior to elution. On reapplying the vacuum of 10 kPa, the methanol was drawn through the columns into the collection tubes. After addition of 0.5 ml of methanol the extraction was repeated and the collected combined eluates were evaporated to dryness under nitrogen at 25°C. The sample residue then was reconstituted in 1.00 ml of mobile phase, and 50 μl of this solution were injected into the liquid chromatograph.

Tissues. Portions of 100 mg of minced tissue

were homogenized with a mixture of 1 ml of ethanol and 1 ml of 0.1 *M* hydrochloric acid using a Potter apparatus. Subsequently the tissue homogenate was vortex-mixed for 30 min and after centrifugation at 2000 *g* for 5 min aliquots of 400 μl of the clear supernatant were diluted with 0.9% sodium chloride solution to 1.00 ml and further processed as described for the extraction of plasma or urine samples.

Quantitation. The concentration of pentisomide and desisopropylpentisomide in plasma, urine and tissues was determined from calibration curves of peak-area ratios (pentisomide and desisopropylpentisomide to internal standard) *versus* pentisomide and desisopropylpentisomide concentrations in plasma, urine and tissue standards subjected to the described procedures.

RESULTS

Typical chromatograms of a reference standard of pentisomide and desisopropylpentisomide and internal standard in mobile phase and from extracts of blank human plasma, plasma spiked with 2 μg/ml pentisomide and desisopropylpentisomide and plasma from a patient treated with 600 mg of pentisomide per day for two weeks are presented in Fig. 2. Chromatograms from extracts of blank human urine, urine spiked with 2 μg/ml of the drugs, blank heart tissue and heart tissue spiked with 10 μg/g pentisomide and desisopropylpentisomide are shown in Fig. 3. The peaks representing pentisomide, desisopropylpentisomide and the internal standard are symmetrical, baseline-separated and well removed from the solvent front and interfering peaks from biological material. The retention times of desisopropylpentisomide, pentisomide and internal standard were 4.8, 5.7 and 10.8 min, respectively.

The retention times of the compounds under study were quite stable, as demonstrated by a coefficient of variation of less than 3% of the mean of the retention times of these compounds assessed each day during an eight-month period. A number of drugs that may be administered concomitantly with pentisomide were investigated for possible interference in the assay (Table I). These drugs were tested by direct injection of methanolic solutions into the liquid chromatograph and their retention times were compared with those of pentisomide, desisopropylpentisomide and internal standard. With the exception of acebutolol, disopyramide and metoprolol, none of these drugs, even after addition to plasma at therapeutic levels, showed any interference with the pentisomide assay, because of either a different retention time, very little UV absorbance at 258 nm or a low recovery with the assay procedure used. Disopyramide in therapeutic levels of 2–4 μg/ml may interfere with the assay, because the peak co-eluted with pentisomide but these drugs are unlikely to be prescribed together.

Interfering peaks were also observed from the β-blocking agents acebutolol and metoprolol after injection of methanolic solutions of 10 μg/ml, however these interferences were negligible after extraction of the drugs at therapeutic plasma levels of 100–400 ng/ml. In addition, because of dangerous cardiac interactions, combined treatment with these drugs is usually not clinically encountered.

Calibration curves of peak-area ratio *versus* concentration were obtained by analysing plasma and urine standard, respectively, containing pentisomide and desisopropylpentisomide in concentrations varying from 0.10 to 10 μg/ml and from 1 to 50 μg/ml. The calibration curves in plasma were established every week, and standard plasma samples were analysed during the week to validate the method.

In addition, a calibration curve in mobile phase was prepared frequently by direct injection of nine mobile phase standards. The equations of the curves were calculated by least-squares linear regression. For the curves in plasma, urine and mobile phase a good linear relationship was obtained in the concentration range studied for both drugs, with intercepts not significantly different from zero.

The linear regression data for the mean calibration curves of pentisomide and desisopropylpentosomide in plasma, urine and mobile phase are summarized in Table II. The plasma and

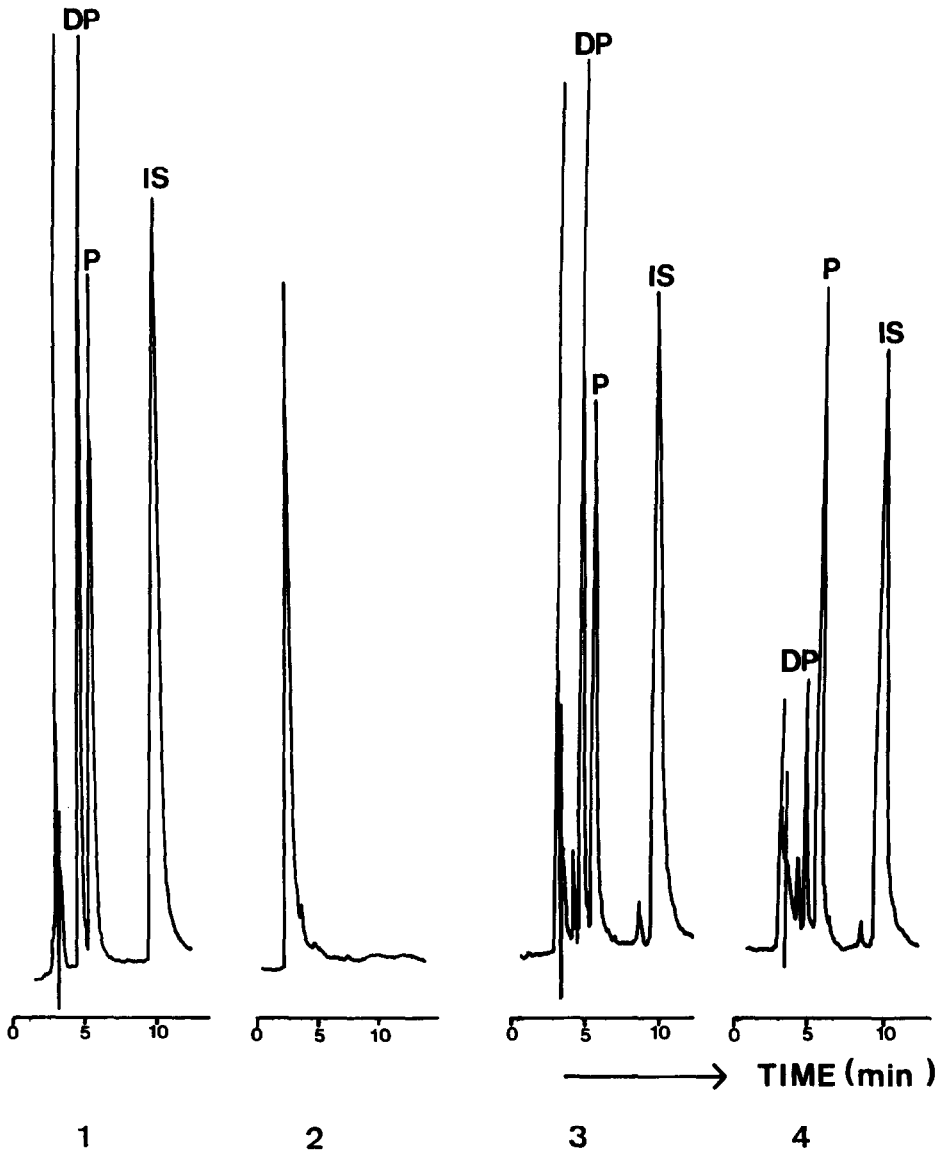

Fig. 2. Chromatograms of (1) reference standard in mobile phase containing 2 μg/ml pentisomide (P) and desisopropylpentisomide (DP), (2) blank human plasma, (3) plasma spiked with 2.0 μg/ml pentisomide (P) and desisopropylpentisomide (DP) and (4) plasma of a patient receiving 600 mg of pentisomide per day for two weeks, containing 2.73 μg/ml P and 0.53 μg/ml DP. Internal standard (IS) concentration: 5.0 μg/ml. Injection volume: 50 μl. Detector sensitivity: 0.005 a.u.f.s. Recorder chart speed: 0.2 cm/mm.

urine curves are the average of eight and three standard curves, which were obtained over a three-month and three-week period, respectively. The mobile phase curves are the mean of five determinations, which were carried out during a three-month period.

The precision of the assay was assessed by replicate analyses of spiked plasma samples containing pentisomide and desispropylpentisomide at concentrations of 0.75, 1.50 and 4.50 μg/ml. The intra-assay and inter-assay coefficients of variation are presented in Table III. The within-day

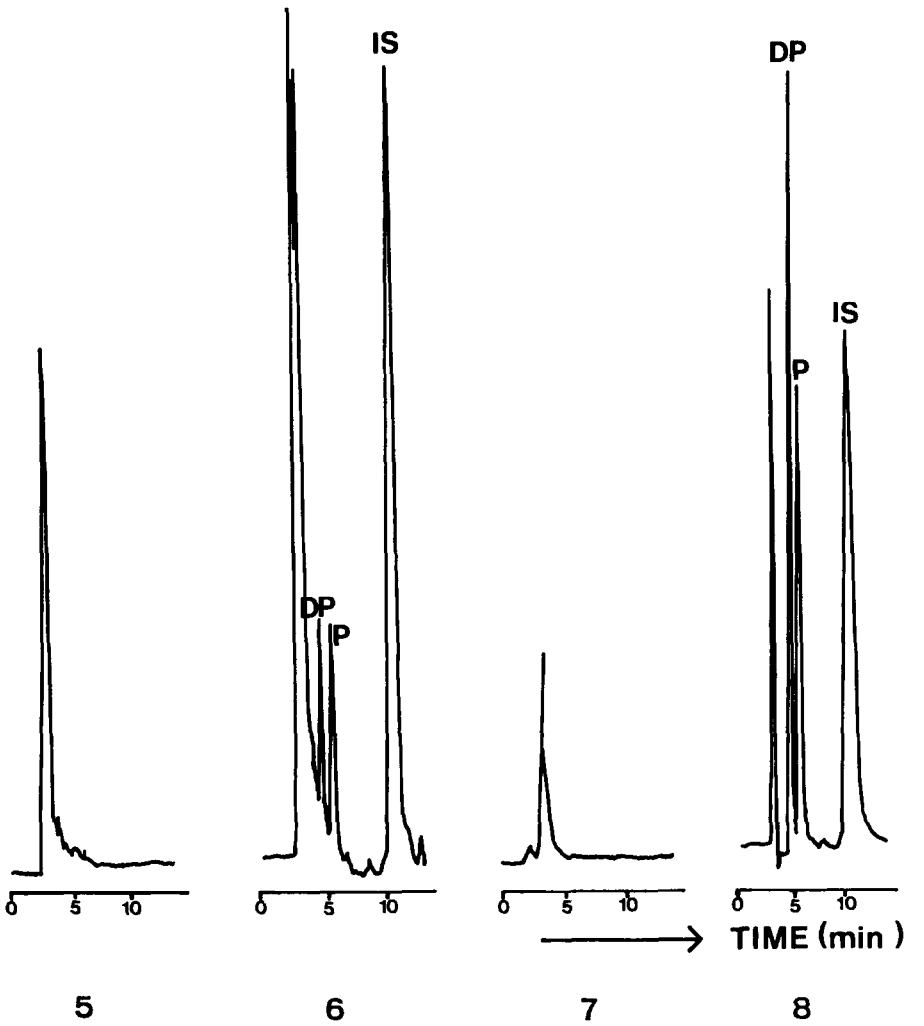

Fig. 3. Chromatograms of (5) blank urine, (6) urine spiked with 2.00 μg/ml P and DP, (7) blank heart tissue and (8) heart tissue spiked with 10 μg/g P and DP. Internal standard (IS) concentration 5.0 μg/ml. Conditions as in Fig. 2.

and day-to-day variation at each concentration for both drugs was less than 8%.

The sensitivity of the assay was 10 ng/ml for both drugs using an injection volume of 100 μl of plasma sample extract. The within-day coefficient of variation per spiked plasma sample containing 0.01 μg/ml was *ca.* 20% (n = 5).

The analytical recovery of pentisomide and desisopropylpentisomide from plasma, urine and various tissues was determined by comparing of the peak areas of pentisomide and desisopropylpentisomide obtained by analysis of 50-μl portions of the mobile phase standards with those obtained from freshly prepared sample extracts. The results of the recovery studies are presented in Table IV. The recovery of internal standard from plasma and urine was 90% (n = 56) and 96% (n = 24), respectively.

The described method was used to determine the plasma levels of pentisomide and desisopropylpentisomide in patients treated with various oral doses of the drug. Blood samples were collected just before the morning dose. The average plasma pentisomide and desisopropylpentiso-

TABLE I

DRUGS TESTED FOR POTENTIAL INTERFERENCE IN THE PENTISOMIDE ASSAY

Drug	Amount injected (ng)	Retention time (min)
Pentisomide	125	5.7
Desisopropylpentisomide	125	4.8
Internal standard	250	10.8
Acebutolol	500	5.6[a]
Acenocoumarol	500	–[b]
Acetazolamide	500	3.6[c]
Acetylsalicylic acid	500	–
Alprenolol	500	22.5[c]
Amiodarone	500	–
Atenolol	500	–
Carbimazole	100	6.1[c]
Chlordiazepoxide	500	24.5[c]
Caffeine	100	5.1
Diazepam	500	–
Dipyridamole	500	4.1[c]
Disopyramide	500	5.4
Flecainide	500	21.2[c]
Furosemide	500	25.6[c]
Hydralazine	500	3.1[c]
Quinidine	100	7.4
Quinine	500	7.8
Labetalol	500	13.1[c]
Lidocaine	500	5.1[c]
Methyldopa	500	–
Metoprolol	500	6.0[a]
Nitrazepam	500	–
Oxprenolol	500	10.3[c]
Procainamide	500	–
Propranolol	500	18.5
Theophylline	500	5.0[c]
Triamterene	500	4.0[c]
Verapamil	500	–

[a] After addition to plasma at therapeutic levels, negligible interference using pentisomide assay.

[b] For drugs with no retention time listed, no peak after 30 min elution.

[c] After addition to plasma; not extracted using pentisomide assay.

mide concentrations in 85 patients receiving a mean oral daily maintenance dosage of 819 ± 229 mg during a mean period of therapy of 3.8 months were 4.58 ± 1.46 and 1.29 ± 0.76 μg/ml, respectively.

In 23 of the patients plasma pentisomide and desisopropylpentisomide levels were assessed at 4–13 days (mean 9 days) during long-term maintenance pentisomide treatment. Small to moderate variations were observed in the individual plasma concentrations of pentisomide and desisopropylpentisomide with coefficients of variations ranging from 7 to 35% (mean 17%) and 6 to 42 (mean 21%), respectively.

The patients had received maintenance dosages varying from 600 to 1200 mg per day for a period of 7–42 months (mean 25 months). Mean steady-state plasma pentisomide and desisopropylpentisomide concentrations with maintenance doses ranging from 600 to 1200 mg per day are summarized in Table V. It should be noted that these mean levels are not completely comparable, since the number of patients in each dosage group was different, which may result in under- or overestimated average values. Mean desispropylpentisomide plasma levels were with all maintenance doses studied clearly lower than the mean pentisomide levels, and the plasma concentration ratio did not increase significantly with increasing maintenance dosages.

In addition, the correlation between the pentisomide and desisopropylpentisomide plasma concentrations (y) and maintenance dose of pentisomide in mg (x) was calculated in 85 patients using least-squares linear regression analysis. A correlation coefficient (r) of 0.54 was found for pentisomide, and the equation of the best-fit line was $y = 1.777 + 0.0034x$. For desisopropylpentisomide a similar correlation was observed ($r = 0.50$) and the expression of the regression line was $y = -0.068 + 0.0017x$. The observed coefficients of correlation (r) indicate a significant ($p < 0.001$) linear relationship between pentisomide and desisopropylpentisomide plasma levels and daily maintenance dosage.

The mean ± S.D. plasma levels of pentisomide and desisopropylpentisomide in fifteen patients on days 3, 15 and 29 after repeated oral administration of 1200 mg/24 h pentisomide are presented in Table VI. The levels in this table show that steady-state concentrations are already attained after 3 days' treatment and no accumulation in

TABLE II

LINEAR REGRESSION PARAMETERS FOR THE MEAN CALIBRATION CURVES OF PENTISOMIDE AND DESISOPROPYLPENTISOMIDE IN PLASMA, URINE AND MOBILE PHASE (MP)

Sample	n^a	Linear regression parameters[b]		Correlation coefficient
		Slope	y-Intercept	
Pentisomide				
Plasma	8	0.2152 ± 0.0146	0.0141 ± 0.0190	0.9995 ± 0.0007
Urine	3	0.2056 ± 0.0015	0.0105 ± 0.0513	0.9993 ± 0.0002
MP	5	0.2043 ± 0.0024	0.0100 ± 0.0025	0.9998 ± 0.0003
Desisopropylpentisomide				
Plasma	8	0.2319 ± 0.0201	0.0020 ± 0.0121	0.9995 ± 0.0005
Urine	3	0.2185 ± 0.0036	0.0149 ± 0.0501	0.9998 ± 0.0001
MP	5	0.2272 ± 0.0018	0.0091 ± 0.0040	0.9998 ± 0.0004

[a] Number of calibration curves used.

[b] Peak-area ratio of drug to internal standard plotted on the y-axis *versus* drug concentration in plasma, urine or mobile phase (in μg/ml) on the x-axis.

plasma was observed for both drugs following a one month therapy.

Plasma concentrations of pentisomide and its metabolite desisopropylpentisomide following oral administration of increasing dosages of the drug and after cessation of therapy are summarized in Table VII. The decrease in pentisomide and desisopropylpentisomide plasma concentration after discontinuation of therapy was used for the assessment of the plasma elimination half-life. Elimination half-lives for pentisomide ranged from 12.57 to 20.61 h (mean 17.45 h) and for desisopropylpentisomide from 9.81 to 19.50 h (mean 15.44 h).

DISCUSSION

The reported chromatographic method for the simultaneous assay of pentisomide and desisopropylpentisomide plasma, urine and tissue con-

TABLE III

PRECISION DATA FOR THE DETERMINATION OF PENTISOMIDE AND DESISOPROPYLPENTISOMIDE IN PLASMA

Concentration (μg/ml)	Coefficient of variation (%)			
	Within-day (n = 5)		Day-to-day[a] (n = 6)	
	Pentisomide	Desisopropylpentisomide	Pentisomide	Desisopropylpentisomide
0.75	6.4	3.0	7.3	5.0
1.50	1.3	2.8	4.4	3.9
4.50	1.3	1.6	4.0	3.5

[a] Analysis performed on six days during a three-week period.

TABLE IV

RECOVERY OF PENTISOMIDE (P) AND DESISOPROPYLPENTISOMIDE (DP) FROM PLASMA, URINE AND TISSUES

Concentration (μg/ml or μg/g[a])	Recovery (%)					
	Plasma (n^b = 8)		Urine (n = 3)		Tissues[c] (n = 6)	
	P	DP	P	DP	P	DP
0.10	89.6	93.1	–	–	–	–
0.25	89.8	89.3	–	–	–	–
0.50	88.3	92.1	–	–	–	–
1.00	94.0	93.9	105.4	106.1	89.5	87.3
2.00	90.5	95.3	103.2	102.7	85.8	84.7
3.00	91.5	93.5	103.6	91.7	89.4	83.9
4.00	89.0	92.9	104.1	90.8	85.6	87.1
5.00	92.2	94.3	95.1	93.8	86.3	83.3
10.00	95.2	101.1	98.0	95.4	79.7	77.7
20.00	90.5	92.6	99.5	95.8	90.3	88.3
50.00	–	–	91.7	96.7	96.0	93.0
Mean	91.1	93.8	100.1	96.6	87.8	85.7
S.D.	2.2	3.0	4.9	5.3	4.7	4.5

[a] Tissue concentration in μg/g.
[b] Number of determinations at each concentration.
[c] Mean recovery data from heart, liver and renal tissue.

centrations using reversed-phase HPLC with UV detection at 258 nm is rapid, sensitive and relatively specific. The Nova-Pak C_{18} 4 μm particle size column in combination with a radial compression separation system (RCSS, Waters Assoc.) and a mobile phase consisting of methanol–water–di-*sec.*-butylamine–phosphoric acid (40:60:0.5:0.2 v/v) pH 3.5 is a highly efficient, reliable, simple and reproducible chromatographic system that may be continuously used for eight months without any apparent loss in column efficiency.

The Nova-Pak C_{18} column was preferred to the conventional Bondapak C_{18} steel column be-

TABLE V

PLASMA PENTISOMIDE (P) AND DESISOPROPYLPENTISOMIDE (DP) CONCENTRATIONS IN PATIENTS WITH CHRONIC VENTRICULAR ARRHYTHMIA ON LONG-TERM PENTISOMIDE THERAPY

n = number of patients; plasma ratio DP/P = plasma concentration ratio DP/P; d = mean duration of treatment in weeks.

Maintenance dose (mg)	n	d	Mean plasma concentrations (μg/ml)		Plasma ratio DP/P
			P	DP	
600	39	8	3.77 ± 1.16	0.90 ± 0.50	0.26 ± 0.13
900	30	19	5.02 ± 0.67	1.50 ± 0.67	0.33 ± 0.17
1200	16	24	5.75 ± 1.05	1.86 ± 0.96	0.34 ± 0.19

TABLE VI

PLASMA PENTISOMIDE (P) AND DESISOPROPYLPENTISOMIDE (DP) CONCENTRATIONS AFTER ORAL ADMINISTRATION OF 1200 mg PENTISOMIDE PER 24 h IN 15 PATIENTS WITH CHRONIC VENTRICULAR ARRHYTHMIA

Duration of therapy (days)	Mean plasma concentrations[a] (μg/ml)		Plasma concentration ratio DP/P
	P	DP	
0	0.0	0.0	–
3	4.94 ± 2.26	1.54 ± 0.95	0.34 ± 0.18
15	4.49 ± 2.51	1.96 ± 1.91	0.43 ± 0.26
29	5.18 ± 2.37	1.94 ± 1.44	0.39 ± 0.27

[a] Plasma was sampled before the morning intake.

cause of its superior economy and convenience, enhanced reproducibility and reliability, high efficiency at lower flow-rates, lower back-pressure and complete elimination of column voiding and channelling.

Our HPLC method has the advantage over the previously reported gas chromatographic (GC) method [13] of a simultaneous determination of the drug and its major metabolite in the same biological sample, whereas the GC procedure is limited to the assay of pentisomide only in plasma and urine. For the gas chromatographic determination of desisopropylpentisomide an additional derivatization step or separate analysis of both compounds would be required, as reported for disopyramide and mono-N-desisopropyldisopyramide, drugs structurally related to pentisomide and its metabolite [16]. The HPLC system employed showed a sensitivity and selectivity superior or similar to previously reported GC and HPLC methods [13–15].

The specificity of the method used, mainly based on the selective C_8 column extraction procedure in combination with the selectivity of UV

TABLE VII

PLASMA PENTISOMIDE (P) AND DESISOPROPYLPENTISOMIDE (DP) CONCENTRATIONS AFTER ORAL ADMINISTRATION OF 600, 800 AND 1000 mg FOR 3 SUCCESSIVE DAYS AND AFTER CESSATION OF THERAPY IN PATIENTS WITH CHRONIC VENTRICULAR ARRHYTHMIA

Dose (mg)	n[a]	Duration of therapy (days)	Mean plasma concentrations[b] (μg/ml)	
			P	DP
600	7	3	3.04 ± 0.85	0.65 ± 0.24
800	7	3	3.57 ± 0.80	0.98 ± 0.43
1000	7	3	4.43 ± 1.61	1.62 ± 0.79
0	7	1	4.04 ± 1.07	1.50 ± 0.75
0	7	3	0.64 ± 0.32	0.17 ± 0.13

[a] n = number of patients.

[b] Plasma was sampled at the moment of drug intake in the morning.

detection at absorbance maximum of the drugs, was clearly demonstrated by the lack of interferences from endogenous material and from 29 drugs possibly concomitantly administered with pentisomide. Therapeutic disopyramide levels may interfere; however, combination of this drug with pentisomide is not usually encountered clinically.

The extraction procedure via Bond-Elut C_8 disposable columns was rapid and simple and required, in contrast to previous methods [13–15], no time-consuming liquid–liquid extraction procedure and centrifugation step. Other solid-phase Bond-Elut columns such as phenyl (PH), cyanopropyl (CN) or octadecyl (C_{18}) tested for applicability in the pentisomide assay showed low and variable recoveries of the drug and/or its metabolite and are therefore unsuitable for this drug assay. The Bond-Elut C_8 extraction columns may be reused at least five times following double washing with methanol after each extraction, without any apparent loss of efficiency.

In our procedure up to ten column extractions may be performed simultaneously in about 12 min. To assess the optimal extraction conditions the order of addition of the solutions to the column, the strength of the sodium carbonate solution used (0.1–0.5 *M*), the volume of acetonitrile (0.5–2 ml) used for clean-up, the applied vacuum for extraction and several elution solvents (methanol, methanol–chloroform, methanol–dichloromethane mixtures) were studied. The best results with respect to accuracy and precision were obtained by first applying a 0.2 *M* sodium carbonate solution to the top of the column and by the washing the column twice with 1 ml of acetonitrile at a vacuum of 34 kPa and by using pure methanol as elution solvent.

The described procedure showed an adequate accuracy and precision, which was indicated by the nearly complete recovery of pentisomide and desisopropylpentisomide from plasma, urine and tissues over the concentration ranges studied (Table IV) and by intra- and inter-day coefficients of variation for replicate analysis of spiked plasma samples of less than 7 and 8%. In addition, for the measurement of levels below 100 ng/ml, the obtained residue, following evaporation of the column eluent to dryness under a stream of nitrogen, may be redissolved in 200 μl instead of 1000 μl of mobile phase.

Our findings concerning mean steady-state levels of pentisomide following long-term oral administration of 900 mg are in good agreement with mean values of 3.25 μg/ml and 3.4–5.4 μg/ml reported by Aliot *et al.* [3] and Gonska *et al.* [5] after the same dose (Table V).

The data on pentisomide and desisopropylpentisomide plasma concentrations after chronic oral administration of 600 mg (Table V) are similar to the mean levels of 3.7 ± 0.5 and 1.0 ± 0.2 μg/ml observed by Walker *et al.* [15]. No comparable data are available on the concentration of pentisomide and desisopropylpentisomide in plasma after repeated oral administration of dosages of 800, 1000 and 1200 mg (Tables V–VII) and on the relationship between plasma levels and daily maintenance dosage. Furthermore, our data indicate that in the steady state plasma levels of desisopropylpentisomide were about 30–40% of the corresponding pentisomide levels with all applied dosages.

Our reported mean elimination half-lifes of 17.5 h for pentisomide and 15.4 h for desisopropylpentisomide are in good agreement with previous reported estimates [4, 11].

Finally, the method could be a valuable tool for the further elucidation of pentisomide disposition and pharmacokinetics. In addition the described method is suitable for routine monitoring of plasma levels of pentisomide as well as its major metabolite desisopopropylpentisomide after intravenous and oral administration.

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