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Note

Stability-indicating high-performance liquid chromatography assay for bepridil hydrochloride drug substance and drug products

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Bepridil hydrochloride (I), β-[(2-methylpropoxy)methyl]-N-phenyl-N-(phenyl-methyl)-1-pyrrolidineethanamine monohydrochloride monohydrate, is a new calcium-channel blocker currently undergoing clinical evaluation for the treatment of angina pectoris. Its antianginal properties have been demonstrated in animal studies[1-5] and in clinical studies[6,7].

Various analytical methods have been reported for determining the bepridil concentration in plasma. These methods are both sensitive and selective and range from gas chromatography (GC) with nitrogen-specific detection[8,9] to GC–mass spectrometry (MS)[10] to high-performance liquid chromatography (HPLC)[11]. These methods focus on very low limits of detection of bepridil in biological media, and thus are not appropriate for assaying drug substance and drug product for product release and stability, since they are not designed to detect and quantitate process impurities and degradation products.

In this paper, a stability-indicating HPLC assay method capable of monitoring the purity of bepridil hydrochloride drug substance and drug product (tablet, capsule and injection solution) is described.

Experiments were performed and data generated establishing the linearity, specificity, ruggedness, precision, sensitivity and accuracy of the method.

EXPERIMENTAL

Equipment

The HPLC system consists of a DuPont (Wilmington, DE, U.S.A.) Model 850 liquid chromatograph equipped with a DuPont automatic sampler, a 20-μl loop, a fixed-wavelength 254 nm detector and a Spectra-Physics (San Jose, CA, U.S.A.) Model 4270 integrator. The column employed is μBondapak™ C_{18}, 10 μm particle size, 30 cm × 4.6 mm (Waters Assoc., Milford, MA, U.S.A.), thermostated at 35°C. A flow-rate of 1.3 ml/min is used throughout the study.

Reagents

HPLC-grade water and acetonitrile, analytical-reagent grade glacial acetic acid (Fisher Scientific, Fairlawn, NJ, U.S.A.) and chromatography grade 1-heptanesulfonic acid sodium salt (Eastman Kodak, Rochester, NY, U.S.A.) are used to prepare the mobile phase. N-Benzylaniline (II) is used as a resolution test compound (Aldrich, Milwaukee, WI, U.S.A.). Bepridil hydrochloride standard and debenzylated bepridil (III) are obtained from McNeil Pharmaceutical (Spring House, PA, U.S.A.). Benzaldehyde and benzoic acid analytical-reagent grade, were used without further purification (Aldrich).

Solutions

Paired ion. Dissolve 1.1 g of 1-heptanesulfonic acid sodium salt in 405 ml of water. Using a pH meter adjust the pH to 2.37 with glacial acetic acid (approximately 15 ml will be needed).

Mobile phase. Acetonitrile–paired ion (580:405).

Sample solvent. Acetonitrile–paired ion (580:405) for drug substance; acetonitrile for drug product.

Standard. Accurately weigh about 37 mg of standard into a 50-ml volumetric flask and dilute to volume with sample solvent.

Drug substance sample. Same as standard.

Drug product sample. Accurately weigh an amount of triturated capsule or tablet granulation theoretically equivalent to 150 mg of bepridil hydrochloride into a 200-ml volumetric flask and add 150 ml of acetonitrile. Shake for 30 min and dilute to volume with acetonitrile. Shake well and filter about 20 ml through prepleated filter paper of 0.22 mm thickness (Schleicher and Schüll, grade 588).
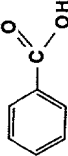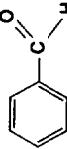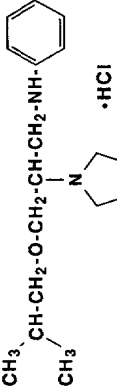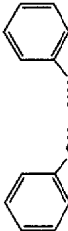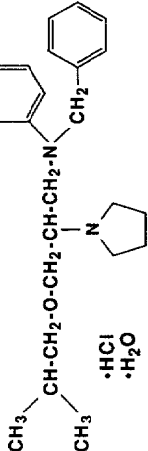
Resolution test mixture. Weigh about 44 mg of bepridil hydrochloride and about 2 mg of debenzylayed bepridil and 10 mg of N-benzylaniline for drug substance and drug product, respectively, into the same 50-ml volumetric flask and dilute to volume with mobile phase.

Diluted resolution test mixture. Accurately dilute a portion of the resolution test mixture in half with sample solvent.

System suitability

Prior to running the system suitability check, the column should be equilibrated for at least 15 min with the mobile phase flowing through the system. An injection of the sample solvent is made to obtain a blank chromatogram. The system suitability is determined by evaluating the resolution solution, injection precision and detector linearity. The resolution mixture is injected and the resolution (not less than 1.6 between peaks) calculated using the standard resolution equation found in the *United States Pharmacopoeia*[12]. The precision is determined using the relative standard deviation of the response factors (area/μg) of injections of the standard solutions. The relative standard deviation (R.S.D.) should be less than 2.0%. An injection of the diluted resolution mixture is chromatographed and the integrated area for the bepridil peak should be within 48–52% of that in the resolution mixture to meet the detector linearity criteria. Acceptable results for system suitability tests are required before samples are analyzed.

TABLE I

STRUCTURE, RETENTION TIMES AND DETECTION LIMITS OF BEPRIDIL HYDROCHLORIDE, ITS PROCESS IMPURITIES AND DEGRADATION PRODUCTS

Compound	Structure	Approximate retention time (min)	Detection limit		Sensitivity factor
			Percent	µg	
Benzoic acid (degradation product of benzaldehyde)		2.6	0.13	0.020	0.17
Benzaldehyde (process impurity)		3.2	0.02	0.003	2.76
Debenzylated bepridil · HCl (III) (process impurity)		4.2	0.09	0.013	0.44
N-Benzylaniline (II) (process impurity)		5.0	0.07	0.010	1.27
Bepridil · HCl (I)		6.8	—	0.01	1.00

Calculations
    To determine percent assay, the following equation is used:

$$\% \ (w/w) \ \text{bepridil hydrochloride} = \frac{R_x P_s}{R_s}$$

where R_x is equal to the response factor of the peak area of the corresponding bepridil peak per μg sample injected, R_s is the response factor of the peak area of the bepridil peak per μg standard injected, and P_s is the percent purity of the bepridil hydrochloride standard. Percent impurities are calculated in a similar manner using a sensitivity factor as described in the Results and discussion section. Unknown impurities are assigned a sensitivity factor of 1.0.

RESULTS AND DISCUSSION

Specificity and stability-indicating ability
    The process impurities and degradation products (Table I) are separated from bepridil hydrochloride and from each other as shown in Fig. 1. The assay value for drug substance in samples spiked with all the impurities listed in Table I at the 3.0% level was not effected (data given in Table II). In the case of drug product, the placebo granulation did not produce any interfering peaks or affect the quantitation of any peaks of interest.

Sensitivity
    The process impurities and degradation products listed in Table I can be quantitated down to at least the 0.2% level for drug substance and drug product. A sensitivity factor was also determined for each impurity. This was accomplished by analyzing samples of these compounds under normal analytical conditions, measuring the resulting peak areas, and dividing by the amount of compound injected. The ratio of the response factors (peak area/μg injected) of the impurity to bepridil hydrochloride is labeled the sensitivity factor in Table I. The sensitivity factor allows the conversion of area percent to weight percent by normalizing the difference in sensitivity between bepridil hydrochloride and the impurity of interest.

Precision
    The precision of the system was evaluated by preparing four portions of the same reference standard and injecting each of them in duplicate. The assay results are given in Table II which show that the relative standard deviation for this set of solutions was 0.22%.

Linearity
    Solutions containing from 0 to 400% of the normal amount of bepridil hydrochloride and from 0 to at least 6.0% of each impurity were analyzed. All calibration plots were linear when using area and intersected near the origin. The regression curve for the bepridil hydrochloride plot gave a slope of 0.603, y-intercept of -0.019 and a correlation coefficient of 0.9999. Regression equations for benzoic acid, benzaldehyde, debenzylated bepridil and N-benzylaniline were $y = 0.097x + 0.001$;
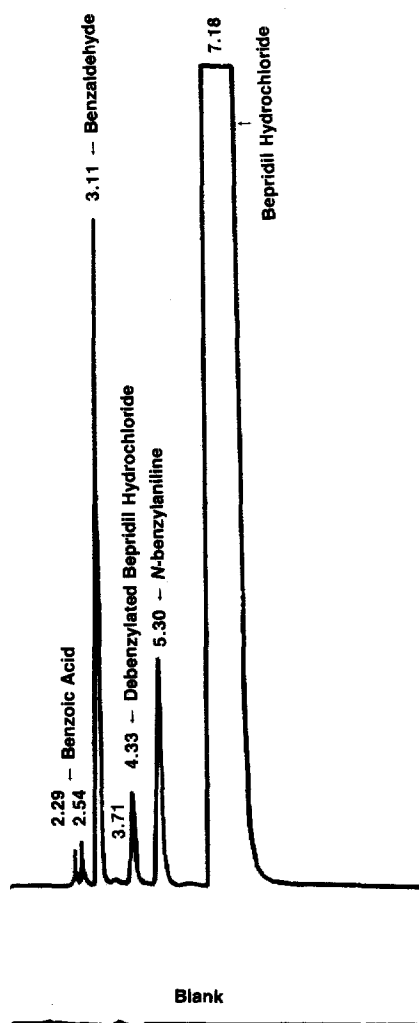
Fig. 1. Liquid chromatogram of the bepridil hydrochloride reference standard spiked with 0.5% levels of benzoic acid, benzaldehyde, debenzylated bepridil and N-benzylaniline using the procedures described herein. Numbers indicate retention times in min.

$y = 1.92x + 0.011$; $y = 0.404x + 0.003$; and $y = 0.917x + 0.001$, respectively. The correlation coefficients were 0.9999 in all cases.

Accuracy

The recovery of bepridil hydrochloride from the drug product was evaluated by spiking placebo granulation with 80% to 120% of the normal amount of bipridil hydrochloride. The recovery of bepridil hydrochloride from these mixtures ranges from 99.2% to 100.1% (Table III).

TABLE II

PRECISION DATA OBTAINED FROM FOUR BEPRIDIL HYDROCHLORIDE REFERENCE STANDARD SOLUTIONS

Sample No.	Beprimil · HCl (mg)	Impurities* (mg)	Recovery (%)**, Bepridil · HCl
1	100.0	0.5	100.5
2	100.0	1.0	100.3
3	100.0	2.0	100.1
4	100.0	3.0	100.0
		Mean	100.2
		R.S.D.	0.22%

* Demonstrates that impurities at concentrations up to 3% do not interfere with the recovery of bepridil hydrochloride.
** The number given is the mean of two determinations per sample.

Ruggedness

The ruggedness of the HPLC system has been demonstrated by obtaining accurate quantitation of bepridil hydrochloride and some potential impurities in a spiked reference standard solution (Table IV) when different flow-rates, column temperatures, columns, mobile phases (pH and composition), instruments and analysts were used. However, benzoic acid and debenzylated bepridil hydrochloride are somewhat affected by the parameter changes.

System suitability

System suitability tests are performed each time the method is run in order to ensure that the entire system is working properly. The resolution is checked by calculating the resolution between the bepridil and N-benzylaniline or debenzylated bepridil in the analysis of drug substance and drug product samples. The linearity of the detector is evaluated by comparing the area of the bepridil peaks of the resolution

TABLE III

RECOVERY DATA OBTAINED FOR BEPRIDIL HYDROCHLORIDE USING THE ASSAY PROCEDURE DESCRIBED

NA = Not applicable; ND = none detected.

Bepridil content (%)		Recovery (%)
Actual	Determined	
Placebo	ND	NA
80% Spike	79.4	99.3
100% Spike	100.1	100.1
120% Spike	119.4	99.5
	Mean	99.6
	R.S.D.	0.34%

TABLE IV

QUANTITATIVE RESULTS (%, w/w) FOR A SPIKED BEPRIDIL HYDROCHLORIDE REFERENCE STANDARD SOLUTION USING VARIOUS CONDITIONS, INSTRUMENTS AND ANALYSTS

Conditions instrument and analyst*	Bepridil · HCl	Benzoic acid	Benzaldehyde	Debenzylated bepridil · HCl	N-Benzylaniline
	Known % (w/w)				
	100.0	0.50	0.50	0.50	0.50
(a)	100.5	0.47	0.55	0.54	0.48
(b)	100.3	0.70	0.54	0.54	0.46
(c)	100.2	0.51	0.53	0.48	0.47
(d)	99.9	0.43	0.51	0.31	0.47
Mean	100.2	0.53	0.53	0.47	0.47
S.D.	0.37	0.12	0.02	0.11	0.01

* (a): Flow-rate 1.3 ml/min, column temperature 35°C, column 1, mobile phase acetonitrile–paired ion solution–acetic acid (580:405:15), instrument 1, analyst 1. (b): Flow-rate 1.0 ml/min, column temperature 40°C, column 2, mobile phase acetonitrile–paired ion solution–acetic acid (580:402:18), instrument 2, analyst 2. (c): Flow-rate 1.5 ml/min, column temperature ambient, column 3, mobile phase acetonitrile–paired ion solution–acetic acid (570:415:15), instrument 3, analyst 3. (d): Flow-rate 1.4 ml/min, column temperature ambient, column 4, mobile phase acetonitrile–paired ion solution–acetic acid (590:398:13), instrument 4, analyst 4.

test mixture and the diluted resolution test mixture. The bepridil hydrochloride concentration of the resolution test mixture is 120% of the normal concentration in order to provide a safety factor for linearity. Finally, the precision is checked by calculating the percent relative standard deviation of the bepridil peak areas of the standards bracketing the samples.

CONCLUSION

The results of thus study indicate that the HPLC method presented is specific, stability-indicating, linear, precise, sensitive and accurate. This method has been found

TABLE V

ANALYSIS OF BEPRIDIL HYDROCHLORIDE DRUG SUBSTANCE FOR RELEASE USING HPLC ASSAY

ND = None detected.

Drug substance lot number	Assay values (expressed as %, w/w)				
	Bepridil · HCl	Benzoic acid	Benzaldehyde	Debenzylated bepridil · HCl	N-Benzylaniline
8506963	100.8	ND	ND	0.07	ND
8304091	100.9	ND	ND	0.08	ND

suitable for the analysis of bepridil hydrochloride, process impurities and degradation products in both drug substance (see Table V) and drug products (tablets and capsules at 100, 200, 300 and 400 mg; injection solution at 4.0 mg/ml.

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