Journal of Chromatography, 421 (1987) 165–170
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3784

Note

Rapid high-performance liquid chromatographic method for the measurement of gallopamil in plasma

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(First received February 19th, 1987; revised manuscript received May 13th, 1987)

Gallopamil, DL-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-2-isopropyl-2-(3,4,5-trimethoxyphenyl)valeronitrile (Fig. 1), is a calcium antagonist which causes specific, reversible inhibition of the transmembrane calcium influx in the myocardium and smooth muscles of blood vessels and uterus, about three to five times more effective than verapamil [1,2]. This compound can be expected to compete with verapamil in treating many angina, hypertensive, and dysrhythmic patients. In order to develop a scientific basis for the design of rational dosage regimens, it is necessary to understand the pharmacokinetics and pharmacodynamics for this drug. To facilitate such studies, one must have a simple, sensitive, and reliable analytical procedure. The method presented here is based on the direct high-performance liquid chromatographic (HPLC) analysis of an extract of a relatively small sample volume using a counter-ion technique.

EXPERIMENTAL

Reagents and chemicals

Gallopamil analytical standard was generously provided by Key Pharmaceutical (Miami, FL, U.S.A.). The internal standard was desmethylimipramine (DMI) in a 0.5 mg/l solution in methanol supplied by Sigma (St. Louis, MO, U.S.A.). Methanol was used for chromatography and reconstitution and was HPLC grade (Burdick and Jackson Labs., Muskegon, MI, U.S.A.). Anhydrous diethyl ether was reagent grade (Fisher Scientific, Fair Lawn, NJ, U.S.A.). Sodium hydroxide 4.0 *M* (Mallinckrodt, Paris, KY, U.S.A.) was prepared from deionized water.

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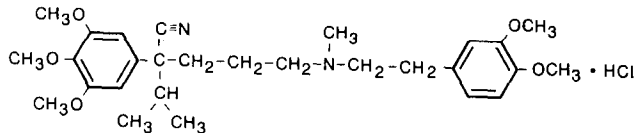

Fig. 1. Structural formula of gallopamil ($C_{28}H_{40}N_2O_5 \cdot HCl$); mol. wt. 521.1 g/mol.

Instrumentation

The solvent delivery system was a constant-flow reciprocating pump (ConstaMetric III, LDC Milton Ray, Riviera Beach, FL, U.S.A.) and sample injection was performed using a Rheodyne Model 7126 syringe loading valve fitted with a 50.0-μl sample loop. The analytical column was a 30 cm$\times$3.9 mm I.D. stainless-steel tube packed with μPorasil, 10 μm silica (Waters Assoc., Milford, MA, U.S.A.), which was used at room temperature. The column effluent was monitored using a Schoeffel Model FS980 fluorescence detector equipped with a 25.0-μl flow cell and utilizing an excitation wavelength of 216 nm; a 320-nm custom bandpass emission filter was employed (Andover, Lawrence, MA, U.S.A.). The mobile phase used for routine analysis was a solution of sodium bromide (0.7 m*M*) in methanol, which was filtered and degassed by sonication prior to use. The flow-rate was 2 ml/min, maintained by a pressure of 100 bar.

The chromatography on this system of a methanolic solution containing gallopamil together with DMI, the internal standard, is illustrated in Fig. 2.

Mobile phase evaluation

To test the chromatographic retention behavior of this adsorption system, we investigated the influence of varying counter-ion concentrations in the mobile phase on the capacity ratios of both gallopamil and the internal standard. The concentrations of sodium bromide used were 0.2, 0.4, 0.6, 0.7, 0.8 and 1.0 m*M* in methanol. For HPLC adsorption systems, retention should be described by a Freundlich-type equation [3, 4]:

$$k' = a[x^-]^b$$

where k' is the capacity ratio, $[x^-]$ is the molar concentration of the counter-ion and a and b are constants.

Emission spectra

Characterization of the emission spectra of gallopamil was carried out on a SPEX Fluorolog 2 spectroflurometer using a Zenon Arch lamp at an excitation wavelength of 230 nm. Selection of the appropriate bandpass filter was based on maximum light intensity.

Validation study

Stock solutions of gallopamil were prepared by dissolving 10 mg of base in 100 ml of methanol. Plasma standards were prepared by adding aliquouts of the stock solutions to human plasma. Standard plasma solutions containing 10.0, 25.0, 50.0, 75.0 and 100.0 ng/ml gallopamil were prepared for calibration curves with 20.0

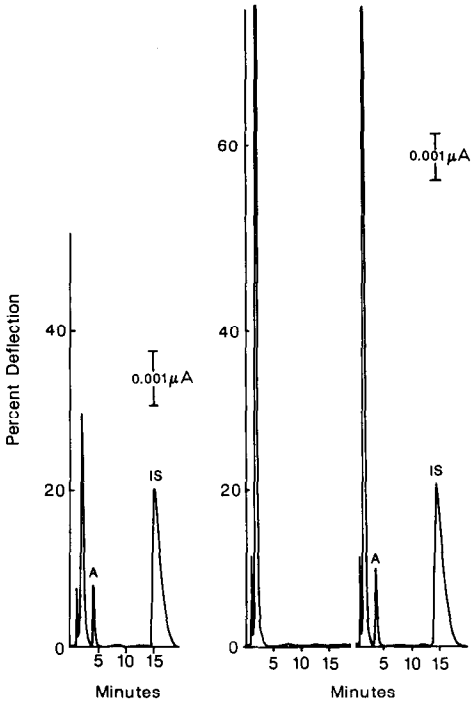

Fig. 2. Chromatogram obtained on analysis of a methanolic solution (left) (20 ng) of gallopamil (A) and DMI, internal standard (IS); drug-free human plasma (middle); and an extracted spiked human plasma standard at 25.0 ng/ml (right). The column is a 30 cm × 3.9 mm stainless-steel tube packed with μPorasil. The eluent is a methanolic solution of 0.7 m*M* sodium bromide.

and 90.0 ng/ml prepared to test accuracy and precision of the assay ($n=10$). Lower limits of detection were determined using methanol standards with the limit of sensitivity defined as a signal-to-noise ratio of 4:1. Percent extraction recovery was determined by comparing peak heights of chromatograms obtained from extracted plasma samples to those of the standard stock methanol solutions at concentrations of 10.0 and 100.0 ng/ml. The stability of 1.5 ml of 10.0 and 100.0 ng/ml standards was determined after storage at −20 °C for 30 days. Potential interfering substances were added to plasma and extracted.

Extraction procedure

Gallopamil was extracted from plasma by a single-step basic extraction procedure. To 0.5 ml of plasma were added 30 μl of a methanolic solution of internal standard. A 4 *M* sodium hydroxide solution (100 μl) was added along with 5 ml of diethyl ether into a round-bottom test tube. The contents of the test tube were vortexed for 1 min and centrifuged at 2000 *g* for 10 min. The ether layer was removed from the original tube by transfer pipette and collected into a glass culture tube, then evaporated to dryness under a nitrogen stream. For HPLC analysis, the extracted residue was redissolved in 100 μl of methanol, and a portion of the reconstituted residue (approximately 90 μl) was injected into the sample loop of the injection valve.

TABLE I

RECOVERY OF GALLOPAMIL FROM PLASMA

Column: 30 cm × 3.9 mm, packed with μPorasil 10 μm silica; mobile phase: sodium bromide in methanol; flow-rate 2 ml/min.

Compound	*n*	Recovery of 10 ng/ml (%)	Recovery of 100 ng/ml (%)
Gallopamil	3	90.7 ± 4.1	94.2 ± 3.7

RESULTS AND DISCUSSION

HPLC conditions

As predicted, there was a linear relationship between k' and Br^- concentration for gallopamil and internal standard ($r = 0.99$, $p < 0.001$). At all concentrations of Br^-, gallopamil had the smallest k' relative to DMI, therefore the shortest retention time.

After characterization of the emission spectra, a plot of relative intensity versus emission wavelengths of gallopamil was made using an excitation wavelength of 230 nm. From these spectra, peak excitation occurred at 318 nm and therefore, a decision was made to use the 320-nm bandpass emission filter to alleviate potential interferences.

Fig. 2 shows typical chromatograms from an extracted blank plasma sample and an extracted spiked plasma standard. The retention times for gallopamil and internal standard were approximately 4 and 14 min, respectively.

Standard curves were prepared by adding known amounts of drug to blank plasma and determining the peak-height ratios (gallopamil peak height/internal standard peak height). The peak-height ratios were then plotted as a function of the concentration of the drug added. Gallopamil standard curves were consistently linear over a range of 10.0–100.0 ng/ml with correlation coefficients ranging from 0.994 to 0.999 and intercept values of −0.02 to −0.09.

Accuracy and precision

The extraction efficiencies of gallopamil at 10.0 and 100.0 ng/ml are shown in Table I. The mean extraction recovery of gallopamil was greater than 90.0% at both concentrations. The coefficient of variation (C.V.) of extraction was less than 5.0% for each determination.

The reproducibility of the procedure was evaluated by analyzing ten replicate samples containing gallopamil on the same day and by analyzing one sample on ten consecutive days. The results are shown in Table II. The C.V. values on replicate gallopamil determinations on the same day and over ten consecutive days were less than 5.0%.

TABLE II

INTRA-ASSAY PRECISION AND REPRODUCIBILITY

Column: 30 cm×3.9 mm I.D. packed with μPorasil 10 μm silica; mobile phase: sodium bromide in methanol; flow-rate 2 ml/min; values given are mean ± S.D. of ten determinations.

Compound	Concentration (ng/ml)	Within-day measured concentration (ng/ml)	C.V. (%)	Between-day measured concentration (ng/ml)	C.V. (%)
Gallopamil	20.0	23.2±0.7	3.0	22.4±0.9	4.0
Gallopamil	90.0	93.8±4.5	4.8	88.8±4.1	4.6

Limit of detection

The limits of detection of the assay determined from methanol standards, defined as a signal-to-noise ratio of 4:1 was 0.9 ng for gallopamil. The limit of detection, in extracted plasma samples, was approximately 1.0 ng/ml.

Stability and interferences

Comparison of peak-height ratios for gallopamil from freshly prepared plasma standards (10.0 and 100.0 ng/ml) to ratios from a sample frozen at −20°C for a period of 30 days showed no significant differences. These data suggest samples drawn for pharmacokinetic analysis can be kept for a minimum of 30 days at −20°C with no degradation.

No endogenous sources of interference have been observed. The chromatogram observed on analysis of a drug-free human plasma specimen is shown in Fig. 2. To assess the potential for chromatographic interference from drugs that are commonly coadministered with gallopamil in the clinical setting, we added and extracted from plasma various cardiovascular drugs. Verapamil, metoprolol, procainamide, N-acetylprocainamide and propranolol were detected if present at high therapeutic concentrations (Table III). Digoxin, diltiazem, disopyramide, lidocaine, phenytoin, quinidine, theophylline, and hydrochlorothiazide were either not extracted and/or not detected.

TABLE III

CAPACITY RATIOS (k') FOR COMPOUNDS COMMONLY COADMINISTERED

Column: 30 cm×3.9 mm I.D. packed with μPorasil 10 μm silica; mobile phase: sodium bromide in methanol; flow-rate 2 ml/min.

Compound	Serum concentration	k'
Gallopamil	10.0 ng/ml	3.5
Verapamil	100.0 ng/ml	4.5
Metoprolol	100.0 ng/ml	8.0
N-Acetylprocainamide	24.0 μg/ml	8.0
Propranolol	100.0 ng/ml	9.0
Procainamide	10.0 μg/ml	12.0
DMI	5.0 μg/ml	14.5

The ion-pair adsorption system presented here proved to be simple, rapid and reproducible. Detrimental effects to the injector, pump, column packing or the detector itself were not observed using the concentration of 0.7 m*M* sodium bromide stated. However, it should be observed that when not required for chromatographic work, we flushed the system with methanol and left it in methanol to avoid crystallization. Under these conditions the system worked properly for more than one year. The useful column life for this analysis is approximately six months. Column equilibration occurs within approximately 1 h.

It is anticipated that this method would be useful in quantitating the polar metabolites of gallopamil. The author has not evaluated the disposition of this calcium channel antagonist following oral administration because it is still an investigational agent in the U.S.A. However, the separation of verapamil, a structurally similar calcium blocker, and its metabolites occurs quite well using this normal-phase system.

CONCLUSION

The procedure reported has sufficient sensitivity for both therapeutic monitoring and pharmacokinetic studies. It is specific for gallopamil. The method is relatively simple and rapid allowing the analysis of 25 samples a day.

ACKNOWLEDGEMENTS

The author wishes to thank Key Pharmaceutical (Miami, FL, U.S.A.) for supplying gallopamil. This work was supported in part by a Research Stimulation Fund Award, Wayne State University (Detroit, MI, U.S.A.)

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