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GAS CHROMATOGRAPHIC DETERMINATION OF GALLOPAMIL AND NORGALLOPAMIL IN HUMAN PLASMA

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SUMMARY

A highly sensitive gas chromatographic assay is described for the simultaneous determination of gallopamil, a calcium channel blocking agent, and its major metabolite, norgallopamil. A multi-step extraction procedure is employed followed by on-column capillary gas chromatographic analysis using nitrogen-selective detection. Acetylation of norgallopamil is performed to enable accurate quantification of the metabolite. Linearity was achieved over the range 1–50 ng/ml for both analytes. Assay specificity, precision and accuracy were investigated.

INTRODUCTION

Gallopamil, a methoxy analogue of verapamil, is a calcium antagonist with a reported activity three to five times greater than verapamil in animal experiments [1]. Studies have shown that similar to verapamil, gallopamil undergoes extensive first-pass metabolism resulting in low plasma concentrations of drug as well as a large number of N-demethylated and O-demethylated metabolites (Fig. 1). The efficacy of gallopamil has been demonstrated in the treatment of angina pectoris. Beneficial effects have also been shown in patients with various cardiac arrhythmias and mild to moderate hypertension [1].

Few papers have been published concerning the analysis of gallopamil in plasma. A gas chromatographic (GC) method for measuring gallopamil with a detection limit of 5.0 ng/ml has been reported [2]. However, since concentrations as low

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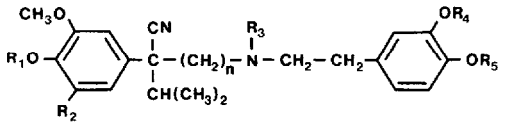

SUBSTANCE	n	R_1	R_2	R_3	R_4	R_5
Gallopamil	3	CH_3	OCH_3	CH_3	CH_3	CH_3
Norgallopamil	3	CH_3	OCH_3	H	CH_3	CH_3
Metabolite I	3	CH_3	OCH_3	CH_3	CH_3	H
Metabolite II	3	CH_3	OH	CH_3	CH_3	CH_3
Metabolite III	3	CH_3	OCH_3	CH_3	H	CH_3
Metabolite IV	3	CH_3	OCH_3	H	CH_3	H
Internal Std	2	CH_3	H	CH_3	CH_3	CH_3

Fig. 1. Structures of gallopamil, several metabolites and internal standard.

as 1–2 ng/ml have been observed in subjects taking therapeutic doses of gallopamil [3], no valid pharmacokinetic parameter estimates can be made using the above approach.

Two high-performance liquid chromatographic (HPLC) methods using fluorescence detection for the simultaneous determination of gallopamil and its major metabolite norgallopamil in plasma have been reported by our laboratory [4] and by Nieder and Jaeger [5]. A more recent publication describes a procedure for the HPLC analysis of gallopamil alone [6]. Each of these methods demonstrated a detection limit less than 1 ng/ml for the analytes.

This paper describes a specific and sensitive GC method enabling the quantification of both gallopamil and norgallopamil with a detection limit similar to HPLC methods.

EXPERIMENTAL

Standards and reagents

Gallopamil (D600), norgallopamil (D845) and five minor metabolites were obtained as hydrochloride salts from Knoll (Ludwigshafen, F.R.G.). The internal standard (Fig. 1), a structural analogue of verapamil (D517), was supplied by Knoll Pharmaceutical (Whippany, NJ, U.S.A.). Stock solutions of gallopamil, norgallopamil and internal standard were prepared in methanol and stored at 5 °C in amber silane-treated volumetric flasks. Methanolic dilutions of gallopamil and norgallopamil used to spike plasma for calibration standards were prepared immediately before use. Working solutions of the internal standard were prepared in water.

All solvents were HPLC grade unless otherwise stated. Pentane, methylene chloride, isopropanol, butyl acetate, methanol, toluene and water were from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Potassium hydroxide and acetic anhydride were supplied by Baker (Phillipsburg, NJ, U.S.A.); hydrochloric acid was obtained from Fisher (Fair Lawn, NJ, U.S.A.).

Glassware

All glassware used to prepare extracts were methanol-rinsed, dried and silane-treated with 10% dichlorodimethylsilane (Kodak) in toluene. Disposable glass culture tubes (16×125 mm) and disposable glass conical tubes (15 ml), both with PTFE-lined caps, were obtained from American Scientific Products (McGaw Park, IL, U.S.A.).

Chromatographic conditions

Analyses were carried out on a Varian Model 6000 gas chromatograph (Varian, Walnut Creek, CA, U.S.A.) equipped with a thermionic specific detector. Operating parameters for the analysis of gallopamil and norgallopamil are summarized in Table I.

Extraction procedure

Plasma (1 ml) and 15 ng of the internal standard in water (100 μl) were pipetted into 16×125 mm silane-treated culture tubes with PTFE-lined screw caps. The plasma samples were adjusted to pH 13 by the addition of 100 μl of 2 *M* potassium hydroxide, then extracted with 5 ml of pentane–methylene chloride–isopropyl alcohol (39:10:1). Mixing was accomplished by rotating the tubes on a Glas-Col rotator (Glas-Col, Terre Haute, IN, U.S.A.) at 75 rpm for 10 min. After centrifugation at 1150 *g* for 10 min, the aqueous layer of the samples was frozen at −80°C in a Revco freezer. The organic layer was poured into a clean silane-treated 16×125 mm culture tube and back-extracted with 2 ml of 1 *M* hydrochloric acid by reciprocal shaking for 10 min on an Eberbach shaker (Eberbach, Ann Arbor, MI, U.S.A.). Following centrifugation at 1150 *g* for 5 min, the samples were frozen at −80°C. After removing the tubes from the deep-freeze, the organic layer was discarded. The samples were made alkaline with 300 μl of 10 *M* potassium hydroxide, then extracted with 5 ml of pentane–methylene chlo-

TABLE I

CHROMATOGRAPHIC CONDITIONS

Column	Fused-silica capillary, 15 m×0.32 mm I.D. DB-1, 0.25 μm film (J&W Scientific)
Injection mode	On-column injector (J&W Scientific) continuous helium purge, 5 ml/min
Injection volume	1 μl
Gas flows	Helium, maintained at constant 30 p s.i.; helium make-up, 30 ml/min; air, 175 ml/min; hydrogen 4.5 ml/min
Temperature	Injector, ambient; detector, 300°C; oven, 130 to 150°C at 35°C/min, 150 to 200°C at 30°C/min, 200 to 300°C at 20°C/min, hold 2 min

ride (4:1) for 10 min on an Eberbach shaker. The samples were again frozen and the organic layer was decanted into a silane-treated 15 ml conical tube. Isopropyl alcohol (250 μl) was added to each tube. The samples were vortexed and evaporated to dryness under nitrogen at 35°C. Acetic anhydride (50 μl) was added to each tube and allowed to stand 15 min. The acetic anhydride was evaporated under nitrogen at 35°C. The samples were reconstituted in 25 μl of butyl acetate. A 1 μl volume was injected for analysis. Peak-height ratios were recorded. Calibration of the data was performed using weighted least-squares linear regression analysis [7].

RESULTS AND DISCUSSION

The development of a GC method for the analysis of gallopamil and norgallopamil presented a unique challenge. Since gallopamil is three to five times more potent than verapamil, lower doses are administered to patients resulting in lower concentrations of parent drug and metabolites.

In order to achieve a quantifiable detection limit of 1 ng/ml for gallopamil, a capillary GC system was developed in order to take advantage of the high resolving power, enhanced sensitivity and inertness of capillary columns. On-column injection was chosen as the technique which would yield the most precise and accurate quantification of low plasma concentrations. A multi-step sample work-up was developed to produce highly pure extracts for analysis.

The quantifiable limit for both gallopamil and norgallopamil was 1 ng/ml. Linearity was achieved over the concentration range 1–50 ng/ml. Intra-assay precision and accuracy were evaluated from the analyses of six plasma samples at three concentrations over the range 1–50 ng/ml for both gallopamil and norgallopamil. Accuracy was within 10% of theoretical at each concentration for gallopamil. The coefficient of variation (C.V.) was $\leqslant$10%. Accuracy values for norgallopamil were within 12% of the theoretical concentrations and the coefficient of variation ranged from 5.7 to 17.3%. The results are summarized in Table II.

Inter-day assay variability was also investigated. The accuracy of the method

TABLE II

INTRA-DAY ASSAY PRECISION AND ACCURACY OBTAINED FOR GALLOPAMIL AND NORGALLOPAMIL IN PLASMA BY CAPILLARY GC ANALYSIS

Compound	Concentration added (ng/ml)	n	Concentration found (mean ± S.D.) (ng/ml)	C.V. (%)	Accuracy (%)
Gallopamil	1.0	6	0.90 ± 0.09	10	90.0
	25.8	6	26.2 ± 2.1	8.0	102
	49.8	6	51.1 ± 2.7	5.3	103
Norgallopamil	1.0	6	0.88 ± 0.05	5.7	88.0
	26.5	6	27.8 ± 4.8	17.3	105
	51.6	6	49.8 ± 3.3	6.6	96.5

TABLE III

INTER-DAY ASSAY PRECISION AND ACCURACY OBTAINED FOR GALLOPAMIL AND NORGALLOPAMIL IN PLASMA BY CAPILLARY GC ANALYSIS

Compound	Concentration added (ng/ml)	*n*	Concentration found (mean ± S.D.) (ng/ml)	C.V. (%)	Accuracy (%)
Gallopamil	1.0	24	0.97 ± 0.12	12.4	98.0
	5.0	12	4.8 ± 0.2	4.2	96.0
	25.8	38	25.8 ± 2.2	8.5	100
	49.8	36	49.6 ± 3.5	7.1	99.8
Norgallopamil	1.0	24	0.92 ± 0.20	21.6	90.6
	5.1	12	5.2 ± 0.4	7.7	102
	26.5	38	27.0 ± 3.9	14.4	102
	51.6	36	48.3 ± 5.2	10.8	93.6

was within 10% of the theoretical plasma concentrations for gallopamil and norgallopamil. Assay precision showed a coefficient of variation ranging from 4.2 to 12.4% for gallopamil and 7.7 to 21.6% for norgallopamil over a similar range (Table III).

The method was shown to be specific for the internal standard and analytes. Fig. 2 illustrates chromatograms obtained from the analysis of blank plasma and plasma spiked with internal standard, gallopamil and norgallopamil. Retention times were 5.4, 5.9 and 6.9 min, respectively. No interference was observed from plasma components. The method was also specific to four known metabolites (I–IV), illustrated in Fig. 1, as well as an N-desalkyl metabolite resulting from the loss of the dimethoxyphenylethyl ring from gallopamil. Extraction studies demonstrated that at a pH of 13, the O-desmethyl metabolites (I–IV) do not extract from plasma. Furthermore, these metabolites do not co-chromatograph with gallopamil, norgallopamil and the internal standard as is illustrated in Fig. 3.

Despite the extensive sample clean-up procedure, routine column maintenance was necessary when chromatographic resolution of peaks deteriorated or significant on-column build-up of plasma components occurred as evidenced by excessive column bleed. To accomplish this 20–30 cm of the injector end of the column was removed. Approximately 2–3 m of the column could be removed without loss of peak resolution.

A common property of amines not often addressed in publications is their ability to adsorb to glass surfaces during the sample work-up [8]. The adsorption tendency of gallopamil was observed in our initial method development during the evaporation step of the sample work-up. In order to prevent substantial loss of drug, isopropyl alcohol was added to the final extract before evaporation of the solvent. Additionally, silane-treated glassware was used as another measure to avoid loss of drug.

Acetylation of norgallopamil with acetic anhydride, a technique used by other investigators [9,10], enabled accurate quantification of this metabolite. Unlike

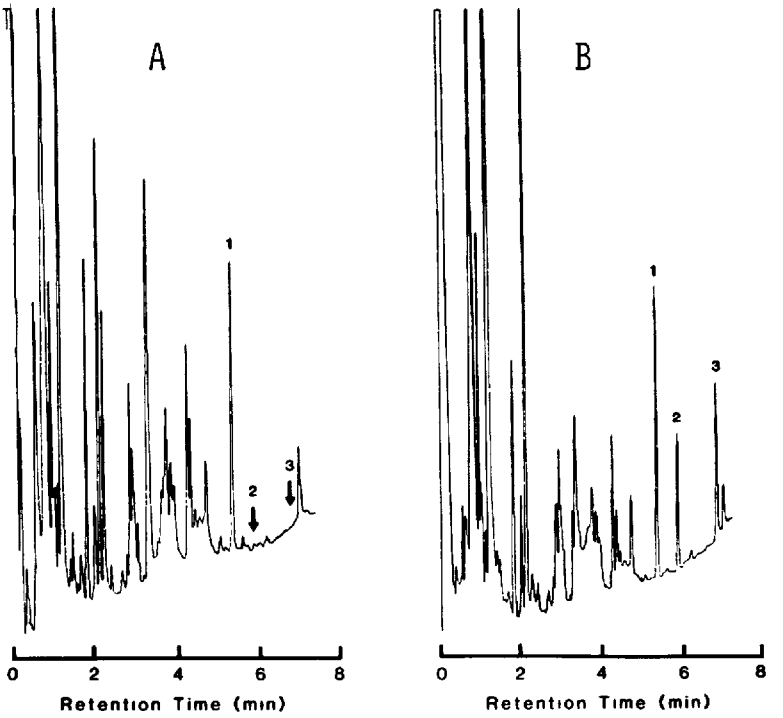

Fig. 2. (A) Chromatogram of blank plasma spiked with 15 ng internal standard (1). (B) Chromatogram of a plasma extract containing 15 ng internal standard (1), 7 ng of gallopamil (2) and 7 ng of norgallopamil (3) per ml of plasma.

gallopamil and the internal standard, underivatized norgallopamil, a secondary amine, is highly reactive to column active sites. Acetylation of this metabolite proved to be a simple technique for conversion of this compound to a less reactive *n*-acetyl derivative. Derivatization of norgallopamil also shifted it away from gallopamil, providing greater resolution between the two components. Fig. 4 illustrates a chromatogram of derivatized and underivatized norgallopamil relative to gallopamil and the internal standard.

We have demonstrated the utility of the assay for measuring gallopamil and norgallopamil in healthy volunteers participating in controlled bioavailability studies. Additional validation of this method is required before it can be used to monitor plasma concentrations in patients who are concurrently taking additional medications. Fig. 5 represents plasma concentration–time profiles of gallopamil and norgallopamil for a subject administered a 5 mg tablet of gallopamil. The half-lives of gallopamil and norgallopamil were 1.9 and 3.2 h, respectively.

This paper presents a sensitive and specific capillary GC method for the analysis of gallopamil and norgallopamil. This approach may be used as an alternative to HPLC methods or as the basis for a GC approach to measuring other metabolites of gallopamil.

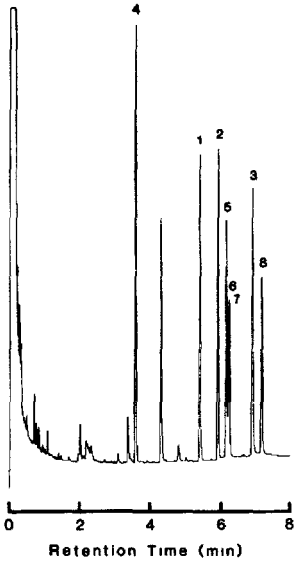

Fig. 3. Chromatogram of a derivatized sample of authentic standards containing internal standard (1), gallopamil (2), norgallopamil (3), N-desalkyl metabolite (4), metabolite II (5), metabolite I (6), metabolite III (7) and metabolite IV (8).

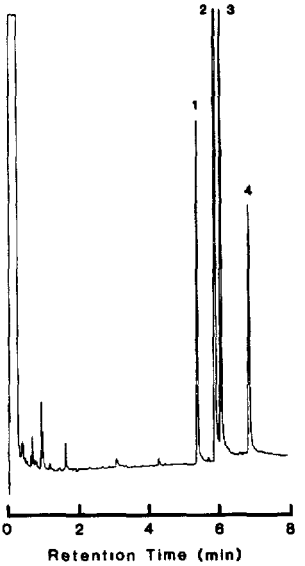

Fig. 4. Chromatogram of internal standard (1), gallopamil (2), underivatized norgallopamil (3) and derivatized norgallopamil (4).

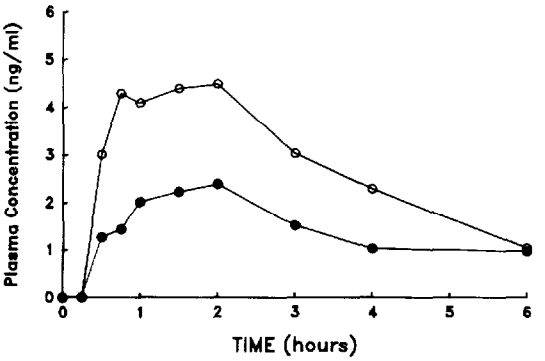

Fig. 5. Plasma concentration–time profile of gallopamil (○) and norgallopamil (●) obtained from a volunteer after administration of a tablet containing 5 mg gallopamil.

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