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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSIS OF PLASMA NIFEDIPINE

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SUMMARY

A gas chromatographic–mass spectrometric assay is described for the determination of plasma nifedipine using nitrendipine as an internal standard Chromatographic separation was performed on a dimethylsilicone capillary column, and detection was by selected ion monitoring of electron impact-generated base peak ions The lower limit of quantifiable detection of nifedipine was 2 ng/ml The method was applied to plasma samples obtained from a human subject who had been dosed with a 10-mg nifedipine capsule every 8 h for eight doses

INTRODUCTION

Nifedipine, a dihydropyridine calcium channel blocker (antagonist), is a potent arterial vasodilator commonly used in the management of angina and other cardiovascular diseases. After a therapeutic dose of nifedipine, maximum plasma drug concentrations are often less than 100 ng/ml and the elimination half-life is less than 2 h in humans [1] Accordingly, pharmacokinetic studies

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of single- and multiple-dose administration of nifedipine require assay methods which can quantitate nifedipine plasma concentrations of 5 ng/ml or less Literature plasma nifedipine assay methods have utilized both high-performance liquid chromatography (HPLC) [2–9] and gas chromatography (GC) [10–18]. In the present investigation, attempts to achieve the required sensitivity and specificity using published HPLC methods were not successful, primarily because of the presence of a minor interfering peak observed in chromatograms obtained from blank plasma samples. This precluded accurate determination of the low plasma nifedipine concentrations (≤ 5 ng/ml) sometimes seen as soon as 4 h after oral administration

Several GC methods have employed less than ideal internal standards [10–15] Of the two mass spectrometric (MS) methods [13,18], Higuchi and Shiobara [18] appropriately utilized deuterated nifedipine as an internal standard However, the method lacks specificity by requiring oxidative derivatization of nifedipine to dehydronifedipine prior to analysis to overcome the variable on-column formation of the dehydrogenated product This same GC oxidation product is reported to be an important metabolite in man [8], present in plasma at concentrations approaching that of the parent drug [10] The oxidation product is also produced by UV light (254 nm) [4] Thus, the method

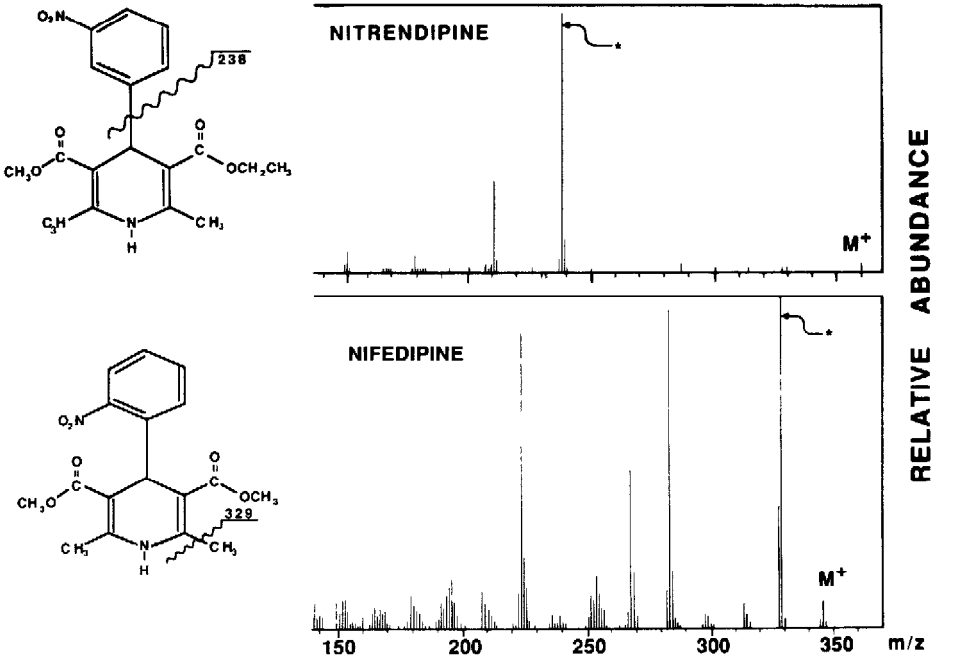

Fig 1 Mass spectra of nifedipine (lower) and the internal standard nitrendipine (upper) The indicated ions (*) were those chosen for selected ion monitoring of plasma extracts The specific fragments corresponding to the indicated ions are depicted on the structures to the left

is subject to yield artifactual nifedipine concentrations corresponding to the sum of the drug, metabolite and any UV degradation product To further confound the interpretation of the chromatography, the nitrosopyridine photodegradation product generated by daylight frequencies (400–600 nm) [4] may also oxidize to dehydronifedipine under the derivatization conditions [19] This latter degradation product is of particular concern because it is formed so rapidly, the half-life of nifedipine in an organic solvent or plasma exposed to daylight is reported to be only 15 and 44 min, respectively [2].

In the present study, use of a dimethylsilicone capillary column overcame the on-column drug instability observed in former studies [1,18], obviating oxidative derivatization Using MS as a detection system resulted in a sensitive and specific GC method applicable to pharmacokinetic studies Structurally related nitrendipine was used as an internal standard (Fig 1)

EXPERIMENTAL

Chemicals and reagents

Acetonitrile, methanol and hexane were obtained from Burdick and Jackson Labs. (Muskegon, MI, U.S A) Isoamyl alcohol, sodium hydroxide and toluene were from Fisher Scientific (Fairlawn, NJ, U S A) Nifedipine was purchased from Sigma (St Louis, MO, U.S.A.). Nitrendipine was extracted with acetone and crystallized from 20-mg tablets (Bayer, Zurich, Switzerland) The metabolites of nifedipine, 3,4-dicarboxy-2,6-dimethyl-4-(2-nitrophenyl)pyridine dimethyl ester and monomethyl ester, were supplied by Bayer (Leverkusen, F R.G.). Chlorotrimethylsilane was obtained from Alfa Products (Danvers, MA, U S A) Nifedipine 10-mg capsules were from Pfizer (New York, NY, U S A)

Collection of human plasma samples

All steps of the analysis including sample collection were performed under gold fluorescent lighting (General Electric Model F40G0 bulbs) to prevent photodegradation of nifedipine [12] A healthy male volunteer was dosed with a single 10-mg capsule formulation of nifedipine every 8 h for eight consecutive doses Blood samples (12 ml) were obtained prior to the final dose and 0 25, 0.50, 0 75, 1, 1 5, 2, 3, 4, 6 and 8 h thereafter. Samples were immediately centrifuged, and the plasma removed and stored in glass vials at $-55°C$ until analyzed.

Preparation of plasma samples for analysis

Aliquots (1 ml) of plasma were placed in 20-ml screw-top centrifuge tubes and 0 05 ml of the nitrendipine internal standard (1 0 μg/ml in methanol) was added to each The tubes were briefly vortex-mixed and then 3 ml of 1 5% isoamyl alcohol in toluene were added The tubes were capped, then shaken on

a horizontal platform shaker for 20 min followed by 5 min of centrifugation at 1800 *g* and 2°C The organic layers were transferred to clean silanized 15-ml conical centrifuge tubes using disposable glass pipets, then evaporated to dryness under nitrogen in a 60°C water bath Acetonitrile (0 1 ml) was added to each tube and the tubes were vortexed for 10 s Acetonitrile-saturated hexane (2 ml) was then added and the tubes were vortexed for 1 min, followed by centrifugation for 3 min at 1800 *g* and 2°C The top hexane layers, which contained endogenous non-polar compounds, were aspirated to waste and the remaining acetonitrile phases transferred to 4-ml disposable glass vials using disposable glass pipets The samples were evaporated to dryness under nitrogen at 60°C, capped and stored at 4°C until analysis, at which time each sample was reconstituted in 10 μl of hexane, and 0 1 μl was injected

Pooled drug-free human plasma was used in preparing standard curves Plasma aliquots (1 ml) were fortified to contain 2 2, 5 5, 16 4, 32 8, 54 7 109 6 and 218 6 ng/ml nifedipine using known concentrations of nifedipine in methanol and then processed with the unknown samples as described above The fitting of standard curves which are composed of a wide range of concentrations, using an unweighted least-squares regression, places heavy weight on the higher concentrations and therefore may introduce a bias Accordingly, the data were fit to an equation of the form $\ln y = a + b\ (\ln x) + c\ (\ln x)^2$, where a, b and c are coefficients [20]. The coefficients were determined from a second-order polynomial fit of a plot of ln nifedipine plasma concentration versus ln drug/internal standard peak-area ratio Nifedipine concentrations for the unknown samples were then calculated using the coefficients from the standard curve and respective peak-area ratios

GC–MS analysis

Instrumentation consisted of a Finnigan Model 9610 gas chromatograph–Model 4000 mass spectrometer interfaced to an IBM-AT computer using a Teknivent Vector/One data system and software (St Louis, MO, U S A) Fragmentation was accomplished by electron impact at an ionizing voltage of 35 eV (70 eV for Fig 1 spectra) with an ionizing current of 250 μA The electron multiplier was operated at 1850 V The instrument was calibrated using perfluorotributylamine The data system acquired two channels of selected ion current: that at m/z 329 for the nifedipine fragment and that at m/z 238 for the nitrendipine fragment The scan rate was every 0 2 s with a sweep width of 0 1 u, integrating each acquisition sample for 4 ms The injector port was adapted for the capillary column using a 17 8-cm conversion sleeve (Supelco, Bellefonte, PA, U S A.) and reducing union Chromatographic separation was accomplished on a 30 m$\times$0.32 mm I D. DB-1 dimethylsilicone fused-silica column (J & W Scientific, Folsom, CA, U S A) The column and injector port were operated at 265°C and the helium carrier velocity was 50 cm/s

RESULTS AND DISCUSSION

The mass spectra of nifedipine and nitrendipine are shown in Fig 1 The relative ion abundances for nifedipine are in agreement with those of Higuchi and Shiobara [18] but differ from those of Jakobsen et al. [13], the latter reporting *m/z* 329 of relatively low abundance. The selection of the base peak ions at *m/z* 329 and 238 for monitoring the drug and internal standard, respectively, maximized sensitivity while providing specificity The two reported plasma metabolites, the pyridine monoester [1,21] and diester [8,21], neither

TABLE I

INTER-DAY AND INTRA-DAY NIFEDIPINE ASSAY PRECISION AND ACCURACY

Theoretical concentration in plasma (ng/ml)	Calculated concentration (ng/ml)				C V (%)
	Day 1	Day 2	Day 3	Mean	
2 2	3 1	2 1	2 3	2 4	15
	2 0	2 3	2 1		
	2 4	2 9	–		
5 5	4 9	4 5	5 0	4 9	16
	5 4	4 1	6 4		
	4 1	–[a]	–		
16 4	17 5	17 1	16 8	16 9	6
	16 5	15 3	16 3		
	18 8	16 5	–		
32 8	29 5	39 5	34 3	32 7	12
	30 4	34 3	27 3		
	33 4	–	–		
54 7	59 1	58 2	52 3	56 2	5
	53 5	55 2	59 5		
	54 8	57 1	–		
109 6	110 0	109 7	113 0	109 2	7
	116 9	99 4	117 8		
	108 2	98 4	–		
218 6	205 2	234 5	202 4	213 9	7
	–	223 6	219 5		
	–	198 2	–		

[a] – = Not determined

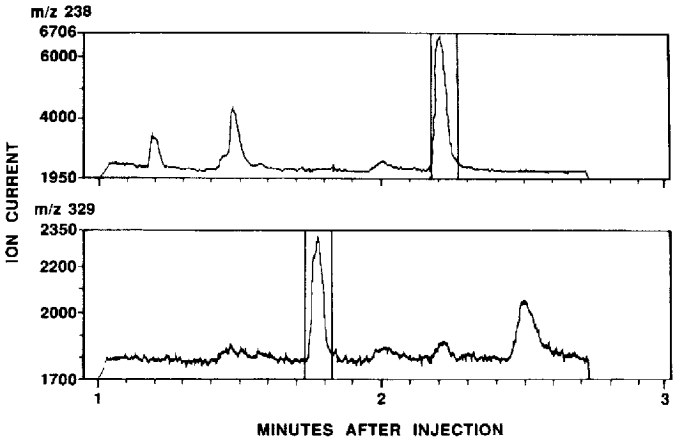

Fig 2 Selected ion chromatogram from a human plasma sample obtained 45 min after the last 10-mg dose of nifedipine (see Experimental) The upper ion profile (m/z 238) corresponds to the base peak ion of nitrendipine (internal standard) and the lower ion profile (m/z 329) corresponds to the base peak ion of nifedipine The pertinent peaks are bounded by vertical lines set by the analyst for peak-area computation This sample was found to contain 16 5 ng/ml nifedipine

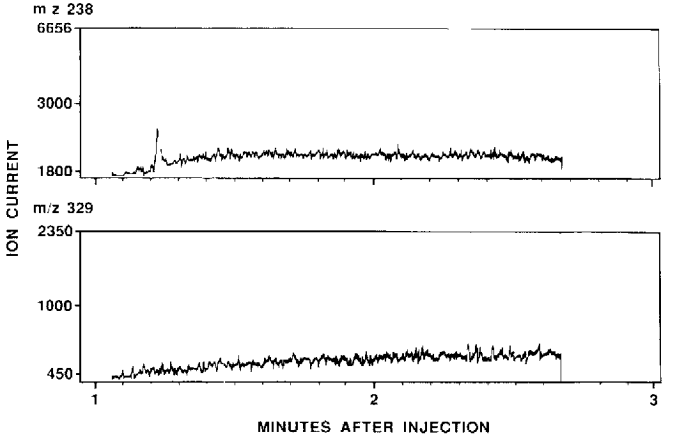

Fig 3 Selected ion chromatogram from blank human plasma sample showing no interfering peak (see Fig 2)

coeluted with nor generated the selected ions common to the drug or internal standard

The intra- and inter-day accuracy and precision of this GC–MS method are summarized in Table I, determined from plasma nifedipine concentrations back-calculated from each of three standard curves prepared on three different days The coefficient of variation ranged from 5 to 16% over the nifedipine concentration range of 2 2–218 6 ng/ml The reliable detection limit for the

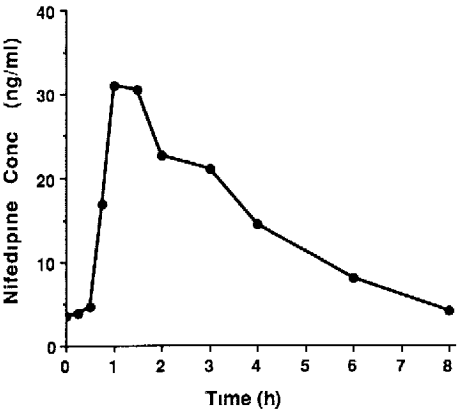

Fig 4 Concentrations of nifedipine in plasma obtained from a healthy male volunteer after the last 10-mg dose (see Experimental)

assay was fixed at 2 ng/ml Correlation coefficients for the standard curves were consistently greater than 0 99

The described GC–MS procedure was applied to the quantitation of plasma nifedipine after multiple dosing in a human subject A typical chromatogram derived from a plasma extract of this subject is illustrated in Fig 2 Identically extracted blank plasma yielded no interfering chromatographic peaks (Fig 3) The plasma concentration–time profile for 8 h after dosing is shown in Fig 4 Because of the rapid clearance of nifedipine, there is very little accumulation of the drug with multiple doses These data indicate that the described assay method is appropriate for pharmacokinetic studies of nifedipine in humans

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