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GAS AND LIQUID CHROMATOGRAPHIC ANALYSES OF NIMODIPINE CALCIUM ANTAGONIST IN BLOOD PLASMA AND CEREBROSPINAL FLUID*

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SUMMARY

Gas (GC) and liquid chromatographic (LC) assay procedures were developed for analysis of nimodipine (1,4-dihydropyridine calcium antagonist, BAY e 9736) in blood plasma at low nanogram concentration levels. To avoid decomposition during gas chromatography, nimodipine was oxidized to nimodipine pyridine (P) analogue before it was chromatographed on the OV-17 column and quantitated using an electron-capture detector. In contrast, the LC procedure involved chromatographic separation and quantitation of the underivatized nimodipine and of the endogenous P analogue using a 3-μm Spherisorb ODS column and UV detection. The same plasma extract and three alternative internal standards were used for both assays. Taking into consideration the fact that the GC assay result includes endogenous P analogue as well as nimodipine, good correlation between GC and LC assay data was obtained. Comparison of the results observed with the two procedures confirmed the accuracy of each procedure and provided an alternative when one of the assay results was subject to patient plasma constituent interference. The LC assay was also used for analysis of the demethylated metabolites of nimodipine. To detect sub-nanogram concentrations of nimodipine in cerebrospinal fluid a combined LC—GC procedure using an LC clean-up step and a GC quantitation step was also developed. The above GC and LC procedures were used to obtain preliminary pharmacokinetic data.

INTRODUCTION

Nimodipine [BAY e 9736, isopropyl-2-methoxyethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate] is a potent calcium

*Dedicated to the memory of Professor Dr. Otto Bayer.

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antagonist which selectively inhibits serotonin- [1] and thromboxane-induced contractions in animal cerebral arteries [2]. In contrast to a structurally related drug, nifedipine, which is used in the treatment of ischemic heart disease, nimodipine yields a marked alteration in cerebrovascular tone without the degree of peripheral vasodilation associated with nifedipine and other calcium antagonists [3–6]. The effect of nimodipine on cerebrovascular tone is most noticeable in the smaller cerebral arteries [7].

Since peroral administration of nimodipine yields relatively low nimodipine plasma concentrations, analytical procedures which can detect nimodipine in blood plasma in the 1—50 ng/ml concentration range are essential. Two independent analytical procedures were developed to meet this requirement. One of these procedures involves gas chromatography (GC) with electron-capture detection which takes advantage of the relatively sensitive response of the electron-capture detector to the nitro group. Similar GC procedures for analysis of the structurally related nifedipine [8—10] and nicardipine [11, 12] were previously reported. As observed earlier [9—12] dihydropyridine compounds are relatively unstable at GC temperatures. Consequently, the GC procedure described in this paper requires oxidation of nimodipine by nitrogen dioxide to the pyridine (P) analogue which does not decompose during GC separation. Although GC assay procedures for analysis of underivatized nifedipine were reported [8, 13, 14], application of similar GC procedures to analysis of underivatized less volatile nimodipine yielded partial (5—20%) conversion during chromatography to the nimodipine P analogue. Furthermore, the observed GC detection limit of nimodipine was about five times higher (less sensitive) than the detection limit of nimodipine P analogue.

In order to assay nimodipine directly without conversion to the P analogue, a high performance liquid chromatographic (LC) procedure was developed. The LC procedure involves separation of nimodipine from the P analogue and demethylated metabolites on a 3-μm Spherisorb ODS column and UV detection of the column eluate.

It was also necessary to develop a procedure which could detect sub-nanogram concentrations of nimodipine in cerebrospinal fluid. An LC clean-up step and a GC quantitation were used for this purpose.

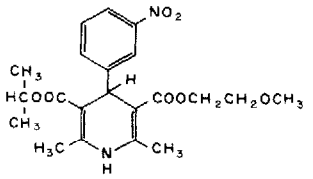
This paper describes the relative accuracy, precision, sensitivity and selectivity of the GC and LC assay procedures. Comparison of the analytical results observed with GC and LC assays of blood plasma obtained during preliminary pharmacokinetic studies is also presented.

EXPERIMENTAL

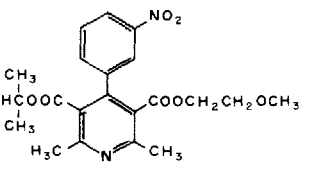
Standards, reagents and solvents

Nimodipine and the appropriate reference and internal standards (see Fig. 1) were obtained from Bayer (Wuppertal, F.R.G.). Stock solutions were prepared by dissolving 10 mg of each substance in 10 ml of methanol or isopropanol and were stored in amber glass volumetrics at 4°C. The mg/ml stock solutions were found to be stable for several weeks but the ng/μl dilutions of the stock solution were freshly prepared every two weeks.

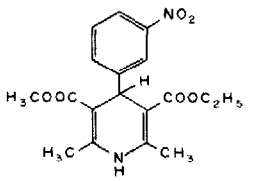
All GC and LC reagents and solvents were analytical or LC grade (Burdick



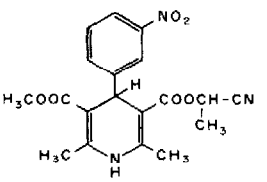
NIMODIPINE



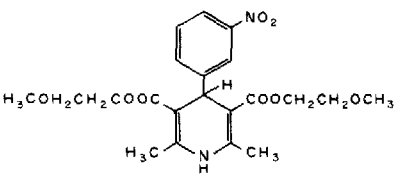
PYRIDINE ANALOG (PA)



NITRENDIPINE



INTERNAL STANDARD B



INTERNAL STANDARD C

Fig. 1. Chemical structure of nimodipine, nimodipine pyridine analogue, and internal standards.

and Jackson Labs., Muskegon, MI, U.S.A.). Diethyl ether was distilled before use. A lecture bottle of nitrogen dioxide was supplied by E. Merck (Darmstadt, F.R.G.) and nanograde toluene by Mallinckrodt (St. Louis, MO, U.S.A.).

Instrumentation and chromatographic conditions

Gas chromatography. GC analysis was carried out on either a Hewlett-Packard Model 5840A gas chromatograph equipped with a ^{63}Ni electron-capture detector and a glass column (1.8 m × 3 mm I.D.) which was packed with 3% SP 2250 DB (Supelco, Bellefonte, PA, U.S.A.) on Gas-Chrom Q, 100–120 mesh (Applied Science Labs., State College, PA, U.S.A.) or a Varian Model 6000 gas chromatograph equipped with a ^{63}Ni electron-capture detector and an Analabs (North Haven, CT, U.S.A.) glass column (1.8 m × 2 mm I.D.) packed with 2% OV-17 on Anakrom Q, 100–120 mesh. Both instruments were operated isothermally at 250–260°C. Carrier gas flow-rate was 25–30 ml/min; argon–methane gas was used with the Hewlett-Packard and nitrogen with the Varian instrument. The Hewlett-Packard chromatograph injection port and detector temperatures were 270°C. The Varian injection port and detector temperatures were 280°C and 320°C, respectively. Sample volumes of 1–2 μl were injected by a Hewlett-Packard 7672A automatic sampling system or a Varian Model 8000 autosampler interfaced with a Spectra Physics SP 4100 (Mountain View, CA, U.S.A.) computing integrator.

Liquid chromatography. A Kratos Model SF 773 (Westwood, NJ, U.S.A.)

UV detector and a Kratos Model 250-1 or Waters Assoc. Model M-45 (Milford, MA, U.S.A.) pump were used for LC analysis. LC samples were injected on an Applied Science Labs. Excalibar (15 cm × 4.6 mm, 3-μm Spherisorb ODS) column using either a Waters Assoc. Wisp 710B or a Perkin-Elmer 1SS-100 (Norwalk, CT, U.S.A.) autoinjector. Two alternative chromatographic solvents were used for column elution. Solvent A consisted of a mixture of acetonitrile—water (57:43, v/v) while solvent B was a mixture of methanol—water (66:34, v/v). Both solvents were pumped at a flow-rate of 0.5 ml/min. The UV detector was set at either 238 nm or 218 nm wavelength, and a 0.004 to 0.002 a.u.f.s. detector sensitivity range. Chromatographic peaks were quantitated using either a Spectra Physics SP 4100 computing integrator or a Waters Assoc. Data Module in the peak area or peak height mode of calculation. Selection of the calculation mode was based on the observed peak shape, resolution and baseline.

Analytical procedures

To prevent UV light decomposition of dihydropyridine compounds to pyridine derivatives during the analysis, all stock solutions of reference and internal standard compounds were stored in amber glassware. Sample extracts were prepared in a room illuminated by either diffuse daylight or shielded fluorescent light fixtures. Since the same initial sample preparation steps were identical, one 3-ml aliquot of plasma was used for preparation of both the GC and the LC sample extracts. The 3-ml aliquot of plasma was mixed with a 3-ml aliquot of an aqueous solution of internal standard prior to extraction with the organic solvent mixture.

Most samples were assayed by reference to one of the three alternative standards. However, if the selected internal standard was not resolved from a plasma sample constituent in a given plasma sample, one of the two alternative internal standards was used for calculation. The internal standard solution was prepared by aqueous dilution of stock ng/μl isopropanol solution of each internal standard in the same volumetric flask to yield a 20 or a 100 ng/ml concentration of each internal standard. To construct the nimodipine and/or nimodipine metabolite calibration curve the concentration of the compound of interest ranged from 10 to 100 ng/ml of the aqueous solution.

Extraction procedure. The plasma sample diluted with the internal standard mixture was treated with 1 ml of 1 *M* sodium hydroxide and extracted once with 17 ml of diethyl ether–hexane (1:1, v/v) solvent during 5 min shaking. The recovery of nimodipine and internal standards (A, B, C) during the extraction was checked and found to be at least 96% for nimodipine, nitrendipine (A) and internal standard B and 92% for internal standard C.

The organic and aqueous phases were separated by centrifugation at 300 *g* for 10 min. One 10-ml aliquot of the organic phase was withdrawn for LC assay while a 5-ml aliquot was used for GC assay. Both aliquots were evaporated under a gentle steam of nitrogen in a 60°C water bath.

Oxidation of GC extract. Test tubes containing residue of the 5-ml GC aliquot were filled with gaseous nitrogen dioxide, sealed, and stored for 1 h in the dark at room temperature. Subsequently, the nitrogen dioxide was flushed out with argon for 5 min at 60°C. Since nitrogen dioxide is toxic, the filling

and flushing of tubes was carried out in a well ventilated hood. Residue in the flushed tube was dissolved in 1 ml of toluene, vortexed and transferred to an autoinjector vial. Normally 1—2 μl of toluene solution were injected onto the GC column.

LC sample treatment. Test tubes containing residue of the 10-ml LC aliquot were washed with about 1 ml of diethyl ether–hexane (1:1 v/v) which was evaporated again under nitrogen. The final residue was reconstituted in 250 μl of modified chromatographic solvent (the chromatographic solvent diluted with water 1:1, v/v). The dilution was necessary because a relatively large sample injection volume (50 μl) was used and the modified sample injection solvent yielded less band spreading (higher column efficiency) than the undiluted chromatographic solvent. A 50-μl aliquot of the reconstituted sample was injected onto the LC column by the Perkin-Elmer or Waters autoinjector. To assay cerebrospinal fluid (CSF) sample by the LC—GC procedure, the LC nimodipine and internal standard fractions were collected and mixed and evaporated prior to nitrogen dioxide treatment of the dry residue and GC analysis.

RESULTS AND DISCUSSION

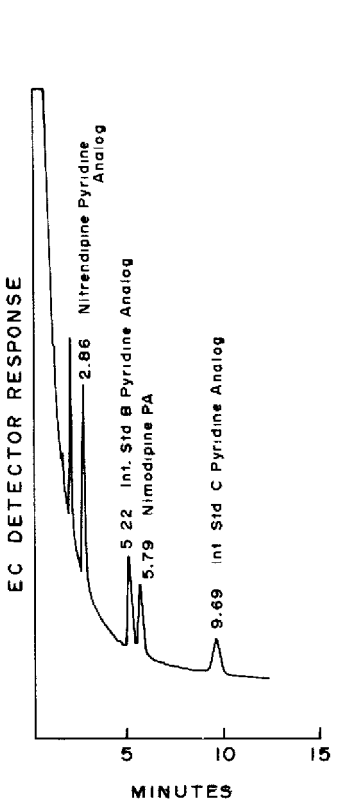
Accuracy and precision

Preliminary results obtained with the GC and LC assay procedures yielded sporadic patient plasma constituent interference in both assay procedures. In spite of the selectivity of the GC electron-capture detector and efficiency of the spherical 3-μm LC column support, some patient samples yielded inaccurate GC or LC results. Since the two procedures involve completely different principles the probability that both assays are subject to the same interference and would yield the same error, is rather small. In view of the above observations the two assay procedures serve as parallel controls to each other.

The sporadic interference could be attributed to unknown patient plasma constituents which were not resolved by the GC or LC column from either nimodipine or internal standard peaks. To minimize the chances of interference three alternative internal standards were used. Thus for all practical purposes, only nimodipine peak interference would impair the accuracy of the assay (the chance that all three internal standards would be subject to interference was small).

Fig. 2 illustrates GC separation obtained with plasma extracts spiked with internal standards and nimodipine reference compounds. In general, internal standard B was used as the internal standard. However, nitrendipine (A) was also used for this purpose when excipient peaks overlapping with internal standard B were observed. Note also that since the sample injected onto the column was oxidized prior to GC, GC nimodipine peak includes endogenous nimodipine pyridine analogue as well as nimodipine oxidized to pyridine analogue. Thus, the concentration of nimodipine determined by GC is a sum of nimodipine and P analogue concentrations present in blood plasma.

Figs. 3 and 4 illustrate LC separations of plasma extracts containing internal standards and nimodipine reference compounds chromatographed on the 3-μm



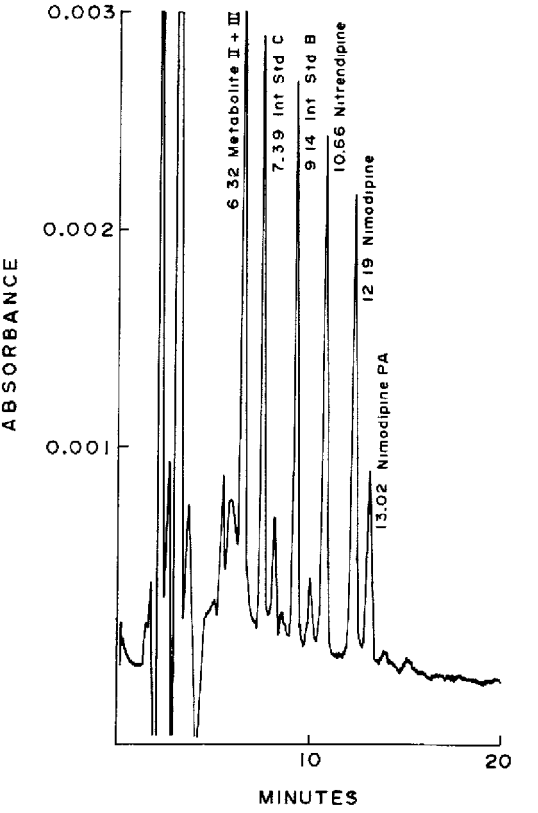


Fig. 2. GC separation of nimodipine and internal standards extracted from plasma and oxidized to pyridine analogues prior to chromatography. Each compound was present in plasma at 20 ng/ml concentration.

Fig. 3. LC separation of nimodipine, nimodipine pyridine analogue, nimodipine metabolites II and III, and internal standards extracted from plasma. Each compound was present in plasma at 20 ng/ml concentration. Spherisorb ODS (3-μm) column was eluted with chromatographic solvent A. The detector was set at 238 nm wavelength, 0.003 a.u.f.s.

ODS column. The same column with two different solvent systems was used to obtain the above chromatograms. Solvent system A was used for analysis of nimodipine and P analogue while solvent system B was used for analysis of nimodipine and two demethylated nimodipine metabolites (M_{II} and M_{III}). Although solvent system A does not resolve M_{II} from M_{III} it was used as an alternative system for assay of some patient samples which were subject to plasma constituent nimodipine and/or P analogue interference.

Figs. 5 and 6 depict the GC and the LC calibration curves. Since the GC assay was used only for analysis of nimodipine after conversion to P analogue while the LC assay was used for analysis of the intact nimodipine as well as endogenous P analogue and two demethylated nimodipine metabolites, the GC calibration required only one calibration curve while the LC calibration required four curves. Linear GC and LC calibration curves were observed within the concentration range of the samples.

Tables I and II present the GC and LC calibration regression line data which

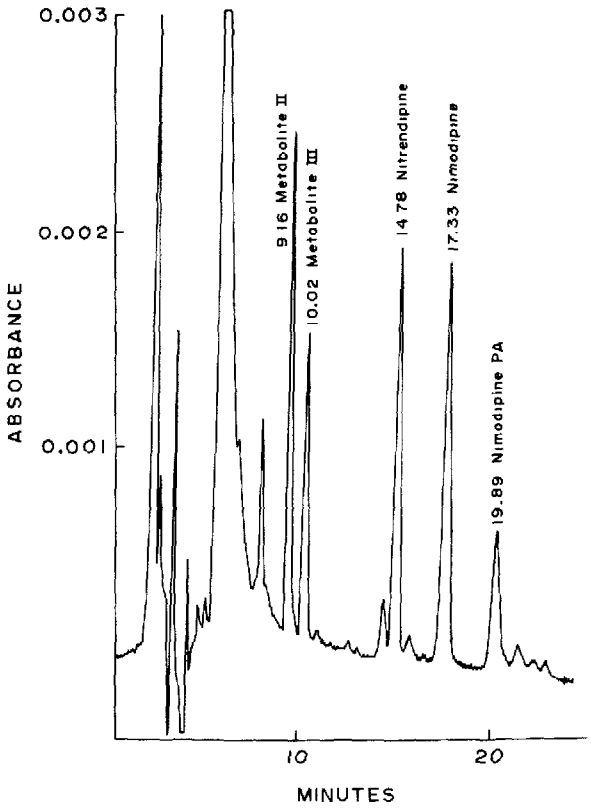


Fig. 4. LC separation of nimodipine, nimodipine pyridine analogue, nimodipine metabolites II and III, and nitrendipine internal standard A extracted from plasma. Each compound was present in plasma at 20 ng/ml concentration. Spherisorb ODS (3-μm) column was eluted with chromatographic solvent B. The detector was set at 238 nm wavelength, 0.003 a.u.f.s.

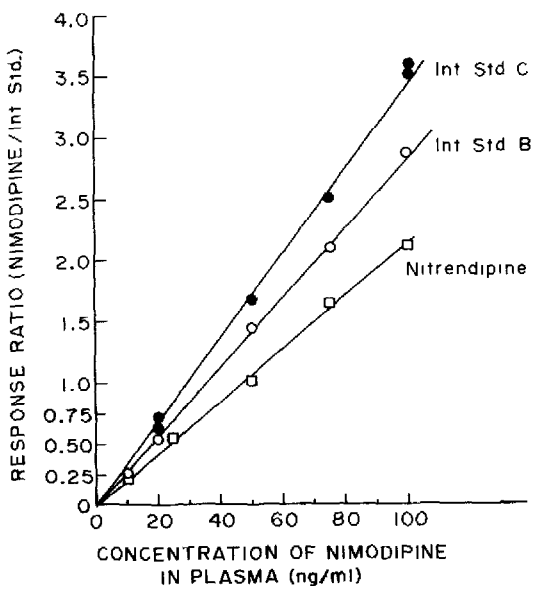


Fig. 5 GC calibration curves of nimodipine obtained by reference to three internal standards.

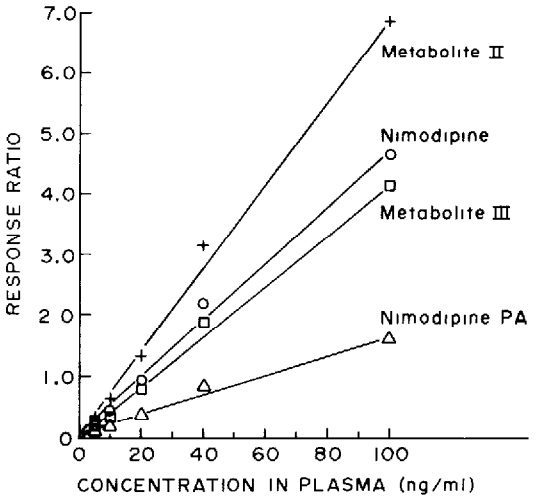


Fig. 6. LC calibration curves of nimodipine, nimodipine pyridine analogue, and nimodipine metabolites II and III, obtained by reference to nitrendipine internal standard A.

TABLE I

GC CALIBRATION CURVE DATA

Peak ratio	Correlation coefficient	Intercept (ng/ml)	Standard error of estimate
Nimodipine/nitrendipine	0.9989	−0.07	1.94
Nimodipine/I.S. B	0.9996	1.11	1.10
Nimodipine/I.S. C	0.9983	2.35	2.41

TABLE II

LC CALIBRATION CURVE DATA

Peak ratio	Correlation coefficient	Intercept (ng/ml)	Standard error of estimate
Nimodipine/nitrendipine	0.9965	0.05	0.18
Nimodipine/I.S. B	0.9926	−0.09	1.32
Nimodipine/I.S. C	0.9996	−1.60	0.28
PA/nitrendipine*	0.9925	0.05	0.09
M_{II}/nitrendipine	0.9970	0.03	0.24
M_{III}/nitrendipine	0.9975	−0.03	0.13

*PA = pyridine analogue.

reflect the accuracy of the GC and LC assay procedures. In general, GC quantitation involved nitrendipine (A) or B internal standards while LC quantitation was based on either internal standard A or C. However, because M_{III} was not resolved completely by LC from internal standards B and C, LC quantitation of M_{II} and M_{III} demethylated nimodipine metabolites was based on internal standard A.

Table III presents the short term (within a day) and long term (between

TABLE III

SHORT- AND LONG-TERM PRECISION OF GC AND LC DATA

Assay	Internal standard	Short-term*				Long-term**			
		n***	Mean	S.D.	R.S.D.(%)	n***	Mean	S.D.	R.S.D.(%)
GC	Nitrendipine	14	0.390	0.016	4.68	14	0.414	0.027	6.63
	C	14	1.697	0.097	5.71	14	1.615	0.093	5.75
LC	Nitrendipine	11	27.2	1.68	6.20	14	28.0	1.400	5.10
	C	11	35.8	1.34	3.85	17	36.2	2.300	6.30

*Within a day.
**Between different days.
***n = number of determinations.

different days) GC and LC precision data. The results presented in Table III are based on peak height determinations. The data presented indicate no significant differences between the GC and LC assay precision. As expected, in general, the single-day assay results are slightly more precise than the assay results obtained on different days.

Selectivity of the GC and LC procedures

The above GC and LC separations illustrate the relative selectivity of both procedures. Both chromatographic procedures resolve compounds of interest from the internal standards and plasma constituents. However, because of chemical instability at GC temperatures only the LC procedure is suitable for quantitative analysis of underivatized nimodipine and the demethylated nimodipine metabolites. Injection of underivatized nimodipine on the GC column using the GC conditions specified in the present procedure yields 20—60% conversion to P analogue. Recent results indicate that decomposition of nimodipine during GC chromatography can be reduced to 5 20% at a higher gas flow-rate and lower injector and column temperature but the detection limit of nimodipine under these conditions is reduced from 1 to about 5 ng/ml.

Apparent decomposition of a structurally related dihydropyridine compound during GC was observed by GC—mass spectrometric (MS) analysis [11]. To circumvent this complication the selectivity of the GC assay was enhanced by use of a thin-layer chromatographic (TLC) separation step prior to GC analysis [12]. The present LC procedure provides an alternative to TLC separation and GC quantitation.

LC separation was also used to enhance the selectivity and sensitivity of the cerebrospinal fluid assay and for detection of the demethylated metabolites of nimodipine which could not be detected by the present GC procedure. Two demethylated metabolites of nimodipine were observed in serum [15, 16]. Both metabolites were detected, resolved, and quantitated by the LC procedure.

Sensitivity of the GC and LC procedures

Based on the signal-to-noise ratio of the GC and LC chromatograms the

detection limit of the procedures described above is about 1 ng/ml. The GC procedure as written specifies injection of 1—2 μl of 1 ml toluene solution. In principle, the GC detection limit could be decreased to 0.2 ng/ml if a smaller volume of toluene and/or a larger injection volume were used. However, because of extraneous peaks, electron-capture detector overload and concurrent enhancement of signal-to-noise ratio, the ultimate practical detection limit of the GC assay is about 0.5 ng/ml for relatively clean sample extracts. Similar limitations apply to potential enhancement of the LC assay sensitivity.

A more effective approach to enhancement of GC and LC assay detection sensitivity involves a combination of an LC clean-up step with a GC quantitation step. This procedure was developed to detect sub-nanogram concentrations of nimodipine in CSF. The effectiveness of this approach is based on the fact that the high capacity and selectivity of the LC column facilitates preparation of a relatively pure nimodipine fraction. This fraction can be concentrated and reacted with nitrogen dioxide in the absence of extraneous compounds to yield pyridine analogue derivative. An external and/or internal standard technique was used to correct for possible partial loss of nimodipine during LC chromatography and nitrogen dioxide reaction. The above approach is also applicable to analysis of samples from the terminal phase of drug elimination. Drug concen-

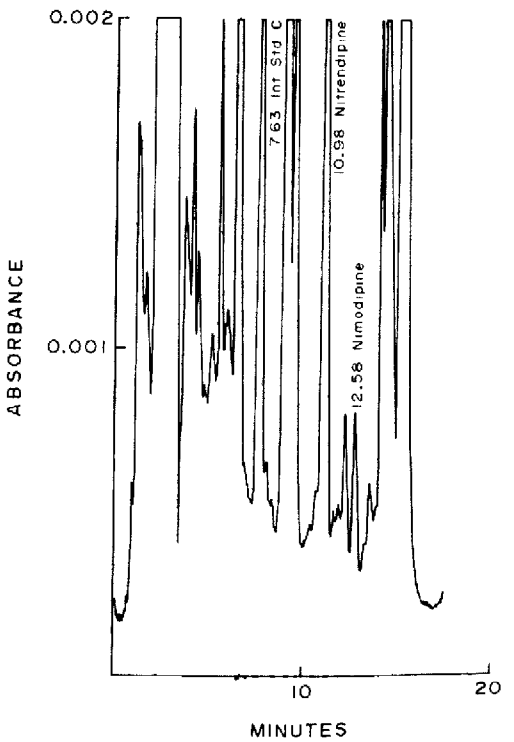


Fig. 7. LC separation of patient CSF extract containing about 0.3 ng of nimodipine per ml of CSF. Nitrendipine and internal standard B were added to CSF prior to extraction to yield 5 ng/ml concentration. The sample injected onto the column was derived from a 2-ml aliquot of CSF. Spherisorb ODS (3-μm) column was eluted with chromatographic solvent A. The detector was set at 238 nm, 0.002 a.u.f.s.

tration in such samples is usually below the detection limit of the direct GC and LC assays.

Some patient CSF samples containing less than ng/ml concentration of nimodipine were estimated directly from the LC chromatogram obtained at high detector sensitivity setting (see Fig. 7). However, subsequent GC analysis of the nimodipine LC fraction enhanced the selectivity, sensitivity, and accuracy of the nimodipine determination. For additional confirmation, some CSF extracts were also analyzed by high resolution, direct inlet MS after LC purification. MS data were quantitated using a multiple ion detection technique based on a deuterated nimodipine internal reference standard.

Table IV presents the CSF results observed by the direct LC, LC—GC and the LC—MS assay procedures. Since each of the above assays is subject to 0.1—0.2 ng/ml error the data presented in Table IV agree within experimental error. It is also of interest to note that the observed concentration of nimodipine in CSF is 10—20 times lower than the concentration of nimodipine in plasma. Since nimodipine is about 95% protein-bound [17] the observed concentration of nimodipine in CSF is approximately equal to the concentration of the free (unbound) nimodipine fraction in plasma. A similar relationship between CSF and plasma concentration of diphenylhydantoin was reported [18, 19].

TABLE IV

COMPARISON OF CEREBROSPINAL FLUID ASSAY BY LC, LC—GC AND LC—MS PROCEDURES

Sample No.	Assay results (ng/ml)		
	LC	LC—GC	LC—MS
621-1	0.3*	0.2	0.3
621-2	0.8	0.8	0.6
644-1	N.P.**	1.0***	0.4
644-2	0.7	0.8	0.4
657-1	N.P.	0.8	0.9
657-2	0.8	0.6	0.8

*2 ml of CSF were used to obtain this estimate.
**N.P. = Not possible to estimate.
***Interference.

Clinical and pharmacokinetic assay results

The GC and LC procedures were applied to analysis of blood plasma collected from patients enrolled in sub-arachnoid hemorrhage and migraine clinical trials and preliminary pharmacokinetic studies in normal volunteers. Statistical analysis of the analytical data collected from patients indicates that there is a correlation between the GC and the combined (nimodipine plus P analogue) LC assay results. Analysis of 80 clinical study plasma samples by the GC and the LC procedures yielded a correlation coefficient of 0.943.

Table V presents preliminary pharmacokinetic study GC and LC assay results. The GC and the combined LC assay results observed with pharmacokinetic data yielded a correlation coefficient of 0.999. It is also of interest to

TABLE V

PRELIMINARY PHARMACOKINETIC STUDY; GC AND LC ASSAY RESULTS BASED ON ONE NORMAL VOLUNTEER

GC vs. LC: Correlation coefficient = 0.999; standard error of estimate = 3.01; intercept = 0.94; slope = 1.00.

Compound(s) assayed	Assay	Concentration (ng/ml) at time (h) after drug administration										
		0.25	0.5	0.75	1.0	1.5	2	3	5	8	24	AUC_{0-8}
Nimodipine + PA*	GC	22.9	126.2	177.7	181.6	86.7	47.5	28.4	13.9	5	2	313.6
Nimodipine	LC	11.9	86.8	116.6	130.6	66.2	34.2	19.6	10.9	4.1	2	224.4
PA	LC	7.0	42.9	56.5	52.1	21.7	11.6	7.5	4.1	2.0	2	90.2
Nimodipine + PA	LC	18.9	129.7	173.1	182.7	87.9	45.8	27.1	15.0	6.1	2	314.6
M_{II}	LC	16.8	143.9	200.6	257.7	126.5	73.4	46.8	25.4	10.9	2	455.3
M_{III}	LC	18.5	189.8	282.8	388.0	172.3	82.8	51.5	24.4	8.5	2	569.8

*PA = pyridine analogue.

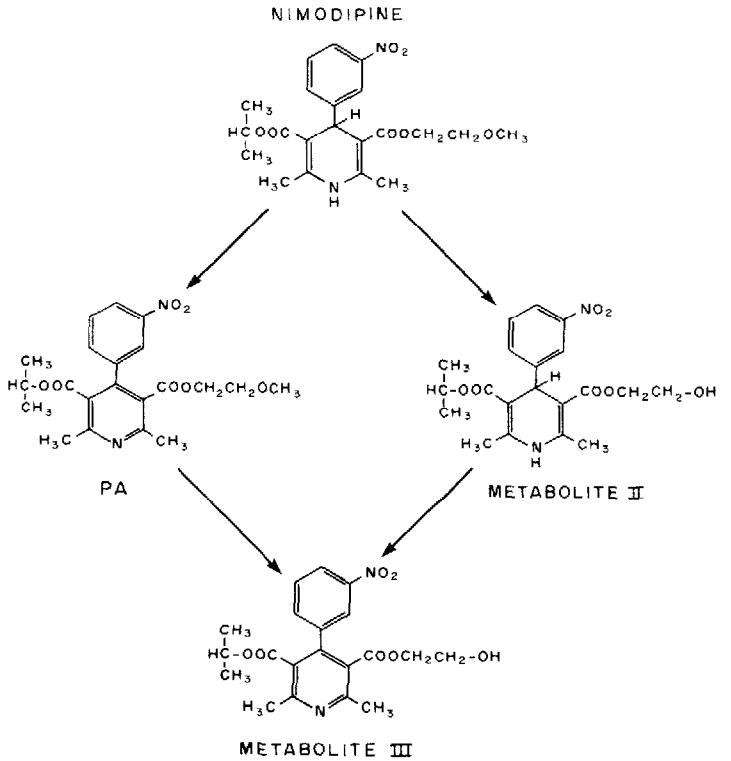


Fig. 8. Initial metabolic reactions of nimodipine.

note that although plasma concentration of nimodipine is considerably greater than the plasma concentration of P analogue, the concentration of the demethylated metabolites is greater than the concentration of nimodipine. The above observation suggests that demethylation occurs more rapidly than oxidation and that the concentration of the demethylated and oxidized metabolite III (M_{III}) is highest because it can form as depicted in Fig. 8 by two sequential reactions. However, the above hypothesis assumes that the elimination and subsequent reaction rates of metabolites observed are similar. It should also be noted that each one of the metabolic products is subject to hydrolysis of each ester side-chain and hydroxylation of methyl groups on the pyridine ring thus the sequence of reactions outlined in Fig. 8 depicts only a fragment of the overall metabolic scheme which was discussed in a previous publication [16]. With the exception of demethylation reaction similar metabolic pathways were proposed to explain nifedipine metabolism [9, 10]. As reported previously the observed dihydropyridine metabolic products are 10 -500 times less potent than nimodipine while the oxidized pyridine products did not exhibit vascular activity [15].

However, the analytical procedures described in this publication are not suitable for analysis of the more polar acidic hydrolysis products of metabolism. Thus, additional work is ongoing to elucidate pharmacokinetics and human metabolism of nimodipine.

CONCLUSIONS

Sensitive and precise GC and LC procedures for assay of nimodipine in patient and volunteer plasma were developed. The relative standard deviation and detection limit of both procedures were about 5% and 1 ng/ml, respectively. Comparison of the results observed with both procedures indicates that the GC and LC data can be correlated providing that the GC results are corrected for the presence of endogenous nimodipine pyridine analogue. Since the LC assay determines nimodipine directly it should be used as a reference method.

Greater selectivity and sensitivity were achieved by combining an LC clean-up step with GC quantitation. This procedure allowed measurement of nimodipine in cerebrospinal fluid and plasma below the ng/ml concentration range. The direct GC, LC and the LC—GC procedures are also applicable to analysis of other dihydropyridine calcium antagonists such as nitrendipine.

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