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Review

Chromatography of calcium channel blockers

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CONTENTS

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LIST OF ABBREVIATIONS

B	Back-extraction
C	Concentration by solvent evaporation
c	Capillary
C_8	Octylsilica
C_{18}	Octadecylsilica
CN	Cyanoalkylsilica
D	Derivatization
E	Liquid–liquid extraction
2E	Repeated extraction
EC	Electrochemical detection
ECD	Electron-capture detection
EI	Electron-impact ionization
FID	Flame ionization detection
Flu	Fluorescence detection
GC	Gas chromatography
LC	Liquid chromatography
LLQ	Lower limit of quantification
MS	Mass spectrometry
NPD	Nitrogen–phosphorus detection
NICI	Negative-ion chemical ionization
P	Precipitation of plasma proteins
p	Packed
PICI	Positive-ion chemical ionization
S	Liquid–solid extraction
SCX	Strong cation-exchanger
Si	Silica
TLC	Thin-layer chromatography

1 INTRODUCTION

Calcium channel blockers are a group of drugs of increasing importance within the cardiovascular field, and their use has developed rapidly during the past ten years. Verapamil was first synthesized in the early 1960s, and diltiazem and nifedipine a few years later, but they were not widely prescribed and used until the mid-1980s. All are now among the twenty most valuable (in money) drugs worldwide. Nifedipine has been followed by more recently developed dihydropyridines with somewhat different pharmacological and therapeutic profiles. Most of these are non-symmetric esters and therefore chiral. Cinnarizine and flunarizine are two other drugs in this group, which so far are less established

This is a fairly new class of drugs, and the measurement of their concentrations in body fluids has been a prerequisite for the documentation needed for new drug applications. Many of the drugs are highly potent, and analytical techniques of high selectivity and sensitivity have been required. Therapeutic monitoring and pharmacokinetic evaluation require that low concentrations of the drugs are determined.

2 GENERAL REMARKS

This review covers gas (GC) and liquid (LC) chromatographic methods; other techniques are left out. This is not a serious limitation since these two techniques are generally superior to others and totally dominate the bioanalytical field with respect to the assay of these drugs.

The papers we have reviewed were with few exceptions published during the 1980s, which means that the methods were developed for modern analytical instrumentation. The GC systems used have changed from predominantly packed column to capillary column technology during this period. As regards the analytical procedures, there are some aspects of common validity.

Solvent extraction is the usual way of isolating the drug from the biological matrix. Most compounds discussed here are highly lipophilic and have sufficiently high distribution constants for a quantitative extraction (>99%) into the organic solvents used Several drugs have a high degree of binding to proteins in the plasma sample, and this may affect the rate and final yield of extraction. Inclusion of the drugs in precipitates from protein denaturation caused by the organic solvent may strongly influence the extraction recovery of lipophilic compounds and the time needed for maximum yield to be obtained.

For highly lipophilic substances liquid–liquid distribution constants may be difficult to determine and are not the only factors determining the recovery from biological samples. Even so, distribution constants may be of significant help in the development of the work-up procedure. In particular, back-extraction into an aqueous phase is more easily optimized if the distribution properties are known. However, published methods often lack liquid–liquid distribution data, as well as data on the influence of other parameters such as the mixing time An extraction recovery significantly below 90–95% will definitely influence the degree of precision and accuracy that can be achieved.

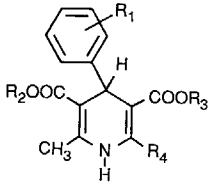
Solutions of highly lipophilic substances cannot be diluted in pure water without the risk of adsorption losses. For amines, 0.01 *M* hydrochloric acid is recommended, and for neutral substances a substantial percentage of organic solvent is required in order to achieve stable dilute solutions. Adsorption losses may also occur with organic solvents of low polarity. These may give reasonable extraction recoveries from plasma but the same solvent may cause losses, *e.g.* in syringes.

In the GC methods, adsorption and degradation of the analyte in the injector, on the column or elsewhere may significantly affect the chromatographic performance in the instrument. Although the symptoms may be noticed, the underlying phenomena are seldom treated in a systematic manner, and more papers that discuss how to eliminate these effects by proper design, selection or treatment of columns and other equipment would be welcome.

In LC, mobile phases without buffers or with very low buffer capacity are often used, although an uncontrolled pH often will affect the retention reproducibility and peak symmetry

TABLE 1

CHEMICAL STRUCTURES OF DIHYDROPYRIDINES



	MW	R_1	R_2	R_3	R_4	Other substituent
Nifedipine	346	2-NO_2	CH_3	CH_3	CH_3	
MPC-1304	388	2-NO_2	CH_3	CH_2COCH_3	CH_3	
Nitrendipine	360	3-NO_2	CH_3	CH_2CH_3	CH_3	
Nimodipine	418	3-NO_2	$CH(CH_3)_2$	$(CH_2)_2OCH_3$	CH_3	
Nisoldipine	388	2-NO_2	CH_3	$CH_2CH(CH_3)_2$	CH_3	
Nivaldipine	397	3-NO_2	$CH(CH_3)_2$	CH_3	CN	
Nicardipine	479	3-NO_2	CH_3	$(CH_2)_2NCH_2C_6H_5$ | CH_3	CH_3	
Benidipine	505	3-NO_2	CH_3	(*R*)-Benzyl-3-piperidinyl	CH_3	
Manidipine	610	3-NO_2	CH_3	2-[4-(Diphenylmethyl)-1-piperazinyl]ethyl	CH_3	
Felodipine	384	2,3-Cl	CH_3	CH_2CH_3	CH_3	
NB-818	457	2,3-Cl	CH_3	$CH(CH_3)_2$	CH_2OCONH_2	
Amlodipine	419	2-Cl	CH_3	CH_2CH_3	$CH_2O(CH_2)_2NH_2$	
Isradipine	371	=N \ O =N /	CH_3	$CH(CH_3)_2$	CH_3	
Flordipine	500	2-CF_3	CH_2CH_3	CH_2CH_3	CH_3	1-[2-(4-Morpholinyl)ethyl]

3. DIHYDROPYRIDINES

Fourteen dihydropyridines (nifedipine, nitrendipine, nimodipine, nisoldipine, nilvadipine, nicardipine, benidipine, manidipine, MPC-1304, felodipine, NB-818, amlodipine, isradipine, flordipine) are dealt with in this review. Their molecular structures are all closely related, the largest variation being in one of the ester groups ($COOR_3$ in Table 1).

3.1. Sample pretreatment

The dihydropyridine structure does not act as either a proton acceptor or a proton donor, and dihydropyridines that do not contain other groups with such properties are neutral over the whole pH range. This is the case for the majority of these compounds. Consequently, pH has no primary effect on extraction recovery. According to published methods, extraction can be carried out without buffers, with buffers at near-neutral pH or under strongly alkaline conditions. This third method may affect the degree of extraction of other constituents in the sample, such as organic acids.

The dihydropyridines are all strongly lipophilic. Very few published data exist on experimentally determined distribution constants for *n*-octanol–water, and those available seem not to be consistent. A theoretical estimation of the relative lipophilicities is presented in Table 2. Nifedipine is here chosen as a reference, and the data refer to the neutral form of the substances. All substances except nilvadipine and amlodipine are more lipophilic than nifedipine The majority of the GC methods for nifedipine employ toluene to extract the substance from plasma. Quantitative recovery can be obtained with this solvent [3], although low and varying recoveries have also been reported [4,5]. In LC methods for nifedipine, more volatile solvents, such as ethyl acetate, chloroform and *n*-pentane–dichloromethane, have been used to facilitate solvent evaporation.

Owing to the relatively higher lipophilicity of the other dihydropyridines, similar or less polar solvent mixtures may be used for extraction from aqueous solutions. However, very hydrophobic dihydropyridines may exhibit slow extraction kinetics. For felodipine, extraction with toluene was complete after 40 min [6].

Nicardipine, benidipine, amlodipine and manidipine have basic properties that may be used to purify extracts further by back-extraction into an acidic aqueous solution. Losses in the batch extraction step may occur, owing to the high distribution constant of the neutral base. Nicardipine was back-extracted quantitatively into 0.05 *M* hydrochloric acid [7]. For benidipine, 1 *M* sulphuric acid was used with high recovery [8]. When amlodipine was back-extracted into 0.1 *M* citric acid, the loss was 40% [9]. Probably a lower pH is required for better recovery (citric acid has a pK_{a1} of 3.1).

Solid adsorbents have been used in few methods for nifedipine [4,5,10]. Bond Elut C_{18} cartridges have been used for nifedipine with higher [5] or lower [4]

TABLE 2

RELATIVE LIPOPHILICITY (Δ log K_D) OF DIHYDROPYRIDINES

Theoretical *n*-octanol–water partition coefficients, K_D, at 37°C and their estimated imprecision calculated according to ref. 1 using data from ref 2 The K_D value for nifedipine is arbitrarily taken as reference

Substance	Δ log K_D	Imprecision
Nifedipine	±0	–
Nitrendipine	0 6	0 2
Nimodipine	0 8	0 2
Nisoldipine	1 6	0.2
Nilvadipine	−0 5	0.4
Nicardipine	1.6	0 4
Benidipine	2.3	0 4
Manidipine	3 4	0 5
MPC-1304	0 1	0 5
Felodipine	2 2	0 2
NB-818	1 2	0 6
Amlodipine	−0 1	0 4
Isradipine	0 3	0 6
Flordipine	2 3	0 4

recoveries than with solvent extraction. Only single applications of solid extraction to dihydropyridines other than nifedipine have been reported. For dihydropyridines exhibiting slow kinetics in liquid–liquid extraction, problems may also be expected in the solid-extraction procedures, where the contact time between sample and adsorbent is short Adsorption onto a charcoal slurry has been used to extract nitrendipine from plasma [11].

Adsorption on alkyl-bonded silica (LiChroprep RP-2) has been used on-line with LC in a closed system, which eliminated sample exposure to daylight [12]. Recovery from plasma samples was the same as for aqueous solutions.

The hydrophobicity of the dihydropyridines makes it difficult to use aqueous solutions for calibration standards and internal standard. Gelatine and boric acid have been used [10] to stabilize aqueous solutions of nifedipine as an alternative to the addition of a miscible organic solvent.

3.1.1. Light sensitivity of nitrophenyl-substituted dihydropyridines

Under the action of daylight and fluorescent light, nifedipine is converted into the corresponding nitrosophenylpyridine. Short-wavelength (254 nm) UV radiation causes oxidation to the nitrophenylpyridine [13]. Several estimates of the degradation rate of nifedipine have been reported [10,14–16]. Nifedipine half-lives in plasma under "normal" laboratory light conditions were between 44 min and 3 h. Evidently the degradation rate depends on the light conditions, which are difficult to specify or standardize. Degradation is even more rapid in organic

solvents [14], and therefore protection from daylight and fluorescent light is essential. Degradation in whole blood is much slower [15] and less of a problem.

Nisoldipine, another 2-nitrophenyldihydropyridine, is also highly light-sensitive [17]. The 3-nitropyridine analogues nimodipine and benidipine also are degraded by light, but at a markedly lower rate [18,19].

3.2. Gas chromatography

The dihydropyridines, which have molecular masses of 346 (nifedipine) and higher, are eluted from GC columns at temperatures ranging from *ca.* 220°C to above 300°C. Their limited resistance to oxidation may lead to conversion into the corresponding pyridine. This was observed in early work on nicardipine [7] and nifedipine [19]. Since the oxidation could not be controlled, the analyte was deliberately oxidized to the corresponding pyridine prior to GC.

Although oxidation is frequently called "thermal decomposition", it will not take place without the catalytic activity of a contacting surface. An oxidizing agent must also be present, but this is normally the case. The amount of oxygen entering a chromatographic system is considerable, compared with the amount of injected dihydropyridine, even with carefully deoxygenated gases. Hot metal surfaces have, not surprisingly, been found to promote oxidation of nifedipine [3]. Hot vaporizing injectors with glass liners, used in the splitless mode, have also caused problems. By changing to split injection [20], solid injection (moving needle injector) [9,21] or cold on-column injection [3,22] oxidation problems have largely been avoided. On the other hand, splitless injection has been used with little oxidation. Lutz *et al.* [23] observed only *ca.* 2% oxidation of nifedipine, including sample pretreatment. This can only be explained by better deactivation of the glass surfaces, although in this case no special precautions were reported to be taken Only a few papers contain information from systematic studies on the GC oxidation of dihydropyridines. Akira *et al.* [24], who used a radioactivity detector to monitor ^{14}C-labelled nifedipine, found that 63.6–75.6% of injected nifedipine was eluted from a packed OV-17 column at the retention time of intact nifedipine. The recovery from an OV-1 column was higher, 72.2–77.8%. By selectively monitoring ions of the formed pyridine, Tokuma *et al.* [25] elegantly showed that nilvadipine may be oxidized, not only in the injector or on the column, but also in the transfer line to the ion source of a mass spectrometer (Fig. 1). Ahnoff [6] showed that the oxidation of felodipine in the injector and on-column (Fig. 2) may be eliminated by treatment with an organic salt, benzyltriphenylphosphonium chloride (BTPPC), or by high-temperature silylation.

GC methods have been developed for all the dihydropyridines reviewed here except manidipine. The high molecular mass (610) of manidipine does not encourage the use of GC. Amlodipine, a primary amine, and NB-818, a carbamate (see Table 1), require derivatization prior to GC. Amlodipine was derivatized with trimethylacetyl chloride after extraction of the drug from plasma and back-

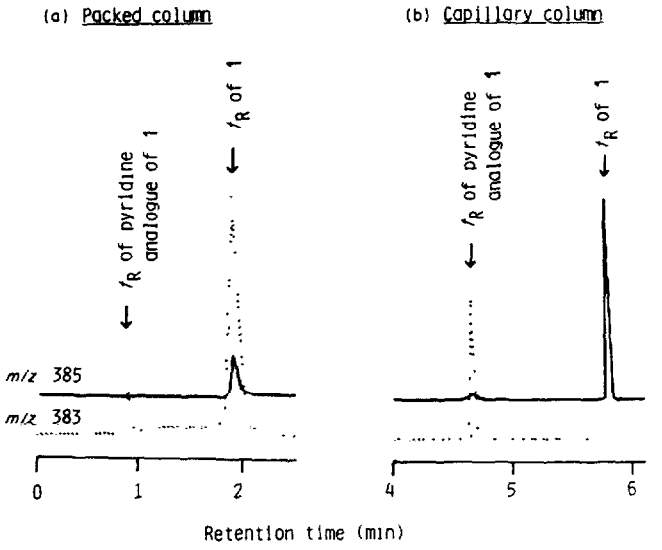


Fig. 1. Selected-ion current profiles of nilvadipine by packed and capillary column GC–NICI-MS; 80 and 20 pg of nilvadipine were injected onto the columns, and ions at *m/z* 385 and 383 were monitored, corresponding to the molecular ions of nilvadipine and its pyridine analogue. Peak heights between these profiles were arbitrary. The capillary column was connected directly to the mass spectrometer. (Reproduced with permission from ref. 25.)

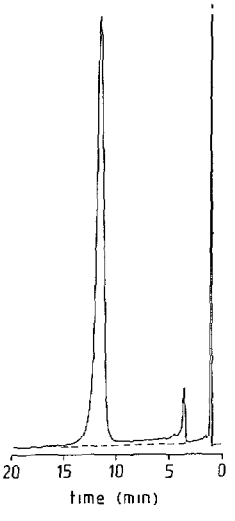


Fig. 2. Chromatogram showing partial oxidation of felodipine (15 ng) injected onto a 2 m × 2 mm I.D. borosilicate column, silanized at room temperature and packed with 3% OV-17 on Gas Chrom Q. The smaller peak and the plateau can be attributed to the pyridine analogue formed in the injector and on the column, respectively. (Reproduced with permission from ref. 6.)

extraction into a citric acid solution. GC of the formed trimethylacetamide derivative was performed on a 5% phenylmethylsilicone capillary column with an elution temperature of 320°C [9].

NB-818 is a dihydropyridine with a carbamoyloxymethyl group in the 2-position. During GC [26] it undergoes elimination of the carbamoyl group and/or abstraction of hydrogen from the dihydropyridine ring, resulting in three degradation products. Therefore, NB-818 was derivatized with a silyl reagent, which replaces the carbamoyl group with a trialkylsilyl group. Dimethylisopropylsilylimidazole was chosen from among other trialkylsilyl reagents on the basis of its reactivity and the properties of the derivative, it withstands an aqueous wash for excess reagent removal, and also exhibits moderately high GC retention (elution temperature 290°C).

3.2.1. Electron-capture detection

The majority of the dihydropyridines have either a 3-nitrophenyl, a 2-nitrophenyl or a 2,3-dichlorophenyl substituent in the 4-position Combined with the dihydropyridine diester structure, this results in a high response in electron-capture detection (ECD), and, with optimized equipment, instrumental detection limits are in the order of 1 pg or less injected (Fig. 3). Consequently, many

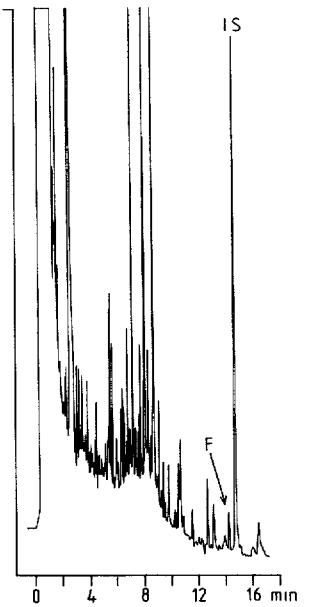


Fig 3 GC–ECD of felodipine (F) and added internal standard (I S) extracted from a patient's plasma with toluene (1:1, v/v) and injected on a 15 m × 0 32 mm I D capillary column coated with 0 25 μm SPB-35 (35% phenyl). The column temperature was 120°C during splitless injection and was thereafter raised to 235°C at 20°C/min The concentrations of felodipine and I S were 0 70 and 12 ng/ml, respectively (2 1 and 36 pg injected). (L. Johannson, unpublished results.)

TABLE 3

CHROMATOGRAPHIC METHODS FOR DIHYDROPYRIDINES

GC or LC	Column	Detection	Pretreatment	LLQ (ng/ml)	Detection limit (ng/ml)	Remarks	Ref
Nifedipine							
GC	p, OV-17	MS, EI	D+2E+C	5		Oxidation with $NaNO_2$	19
GC	p, OV-101	FID	2E+C	300			13
GC	p, OV-17	ECD	E	–	1	Plus pyridine analogue	27
GC	p, OV-1	ECD	D+E+C	5		Oxidation with $NaNO_2$, plus metabolites	28
GC	p, OV-17	ECD	2E+C+E+C	<10		Plus pyridine analogue	29
GC	p, OV-17 or OV-1	ECD	E or S	10–25			4
GC	p, OV-101	ECD	E	1		Plus metabolite	30
GC	c, methyl	NPD	2E+C	2	0.5	Plus pyridine analogue	22
GC	c, SE-30	ECD	E	–	0.5		15
GC	c, DB-1	ECD	E	–	1	Plus metabolites	31
GC	c, DB-5	ECD	E	2		Plus pyridine analogue	23
GC	c, OV-1	ECD	E+C		1	Plus metabolites	3
GC	c, DB-1	MS	E+C+E+C	2		EI *m/z* 329	32
LC	C_{18}	UV 254 nm	E+C	1000		Rat plasma, plus metabolites	33
LC	Si	UV 280 nm	E+C+D+E+C	2		Oxidation with $NaNO_2$	34
LC	C_{18}	UV 235 nm	S+C+E+C	3			10
LC	C_{18}	UV 229 nm	E+C	2			35
LC	C_8	UV 238 nm	E+C	2		Plus pyridine (in urine)	14
LC	C_{18}	EC +0.95 V	E+C		2		36
LC	C_{18}	UV 235 nm	E+C	5			37
LC	C_{18}	EC + 1.0 V	2E+C		2		16
LC	C_{18}	UV 254 nm	2E+C		10	Plus three metabolites	38
LC	C_{18}	UV 355 nm	S	2		Column switching	12
LC	Diol	UV 235 nm	E		1		39
LC	C_8	UV 235 nm	S+C		5		5

Nitrendipine							
GC	c, SE-52	MS, EI	E+C	0 5			40
GC	c, 5% phenyl	MS, NICI	S+C	0.1			11
GC	c, DB-1	ECD	E+C	0.05		Plus pyridine analogue	40a
LC	C_{18}	UV 238 nm	E+C		1		41
Nimodipine							
GC	p, SP-2250 or OV-17	ECD	E+C+D+C		1	Oxidation with gaseous NO_2	42
GC	p, OV-17	ECD	E	0 5			18
LC	C_{18}	UV 238 nm	E+C	1		Plus two metabolites	42
Nisoldipine							
GC	c, DB-1	ECD	E+C	0 05–0 10		Plus metabolite	17
Nilvadipine							
GC	c, 5% phenyl	MS, NICI	E+C	0.010			43
GC	c, 5% phenyl	ECD	E+C	0.25	0 10	Plus pyridine analogue	45
LC	Chiralpak OT	GC–MS, NICI	E+C	0.025		Chiral separation	44
Nicardipine							
GC	p, OV-1	ECD	2E+B+D+E+C	2-3		Oxidation with $NaNO_2$	7
GC	p, OV-1	MS, EI	2E+C+TLC+ D+2E+C	5		Oxidation with $NaNO_2$ after TLC separation from pyridine analogue	46
GC	c, methyl silicone	ECD	E+B+E+C	1		Plus pyridine analogue	20
LC	C_{18}	UV 254 nm	E+B+E+C	5		Plus pyridine metabolite	47
LC	Si	UV 254 nm	E+C	5			48
Benidipine							
GC	p, OV-1	ECD	2E+B+D+2E+C	0 3		Oxidation with $NaNO_2$	8
GC	p, OV-1	ECD	D+2E+C	2		Oxidation with $NaNO_2$	8
Manidipine							
LC	C_{18}	UV 230 nm	E+C		0 1	Column switching	49
MPC-1304							
GC	c, methyl silicone	MS, NICI	2E+C	0 05			21

(Continued on p 192)

TABLE 3 *(continued)*

GC or LC	Column	Detection	Pretreatment	LLQ (ng/ml)	Detection limit (ng/ml)	Remarks	Ref
Felodipine							
GC	c, 5% phenyl	ECD	E	0.8		Plus pyridine analogue	6
GC	c, 0% or 5% phenyl	MS, EI	E+C	0.08			50
GC	c, 35% phenyl	ECD	E	0.4			50
GC	c, 50% and 0% phenyl	ECD	E	0.15		Dual-oven column switching	50
NB-818							
GC	c, 50% phenyl	MS, EI	S+C+S+D+E+C	0.1		Dimethyl isopropylsilyl derivative	26
Amlodipine							
GC	c, 5% phenyl	ECD	E+B+D+E+C	0.1		Trimethylacetamide derivative	9
LC	C_{18}	EC	E+B	0.2			51
Isradipine							
GC	c, methyl	MS, NICI	E+C		0.04	Plus metabolites	52
LC	C_{18}	UV 325 nm	E+C	1		Plus pyridine analogue	53
Flordipine							
GC	p, OV-17	ECD	E+C	2.5			54
LC	C_{18}	UV 238 nm	E+C	25			55

procedures have been designed where the plasma extract is injected without preconcentration (Table 3).

Other dihydropyridines (amlodipine, flordipine) have a lower ECD response, which necessitates preconcentration of extracts. Owing to the high sensitivity of ECD for most of these compounds, nitrogen-selective detection has been little used [22].

3.2.2 Mass-selective detection

In spite of the high sensitivity attainable with ECD for nitrophenyl- and dichlorophenyl-substituted dihydropyridines, its selectivity is not always sufficient at very low levels of the analyte. Higher selectivity may be achieved with mass-selective detection (Fig. 4). The sensitivity of ion monitoring with electron-impact (EI) ionization differs considerably between dihydropyridines, depending on the ionization yield and the fragmentation pattern. For example, higher signal-to-noise (S/N) ratios per injected amount are obtained for felodipine than for nifedipine, as shown in Table 4. In order to exploit the selectivity of detection and obtained high assay sensitivity, samples have to be concentrated to a small volume prior to GC–MS analysis.

Tokuma *et al.* [25,43] showed that higher sensitivity could be obtained with electron-capture negative-ion chemical ionization (NICI) with methane as the reagent gas (Fig. 5), owing partly to less fragmentation, but mainly to higher ionization yields. Also in this ionization mode, S/N ratios varied between differ-

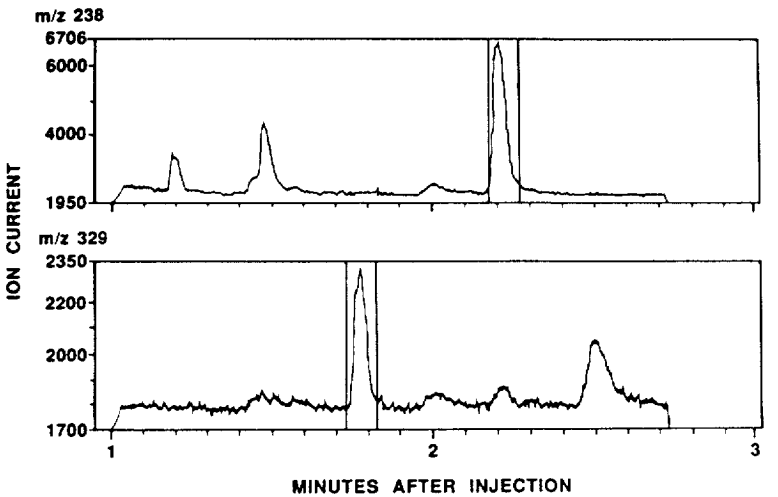


Fig 4 GC separation and mass-selective detection of nifedipine from a human plasma sample containing 16 5 ng/ml nifedipine. The mass spectrometer was operated in EI mode (ionization voltage 35 eV). The upper profile (*m/z* 238) corresponds to the base peak of nitrendipine (internal standard) and the lower profile (*m/z* 329) corresponds to the base peak of nifedipine The fused-silica column (30 m × 0 32 mm I D DB-1 dimethylsilicone) was operated at 265°C (Reproduced with permission from ref 32)

TABLE 4

SENSITIVITY OF MASS-SELECTIVE DETECTION OF DIHYDROPYRIDINES FROM PLASMA SAMPLES

Amounts injected at the detection limit or at the LLQ Data were calculated from the values of LLQ, initial and final sample volume and injected volume, as given in the references

Compound	Mode	Monitored ion (m/z)	Sensitivity	Ref
Nifedipine	EI	298[a] ($[M-46]^+$)	300 pg, LLQ	7
	EI	329 ($[M-17]^+$)	20 pg, LLQ	32
	NICI	346 (M^-)	0 5 pg, S/N = 6	25
Nitrendipine	EI	238 ($[M-122]^+$)	10 pg, LLQ	40
	EI	238 ($[M-122]^+$)	100 pg, S/N > 5	11
	NICI	360 (M^-)	5–10 pg, S/N > 5	11
Nilvadipine	NICI	385 (M^-)	0 5 pg, S/N = 8	25
Nicardipine	EI	134[a]	300 pg, LLQ	46
	NICI	479 (M^-)	5 pg, S/N = 4	25
MPC-1304	NICI	281 ($[M-107]^-$)	2 pg[b], LLQ	21
Felodipine	EI	238 ($[M-145]^+$)	1.8 pg, S/N = 3	7
	NICI	–	5 pg, S/N = 9	25
NB-818	EI	368[c] ($[M-145]^+$)	2 pg, S/N = 9	26
Isradipine	NICI	311 ($[M-60]^-$)	1 6 pg detected	52

[a] Pyridine derivative
[b] Injected with split 20 1.
[c] Dimethylisopropylsilyl derivative

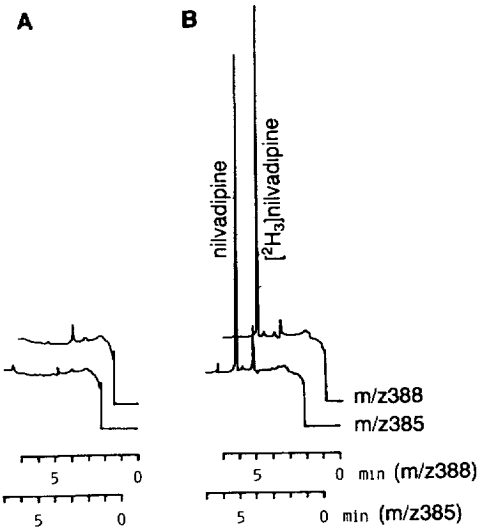


Fig 5 Selected-ion current profiles from GC–MS analysis of HPLC fractions from (A) blank human plasma and (B) human plasma containing (+)-nilvadipine (1 ng/ml) and $[^2H_3]$nilvadipine (2 ng/ml) The column (12.5 m × 0 31 mm I D , coated with 0 52 μm 5% phenylmethylsilicone) was connected directly to the mass spectrometer NICI-MS was carried out with methane as the reactant gas See also Fig 7 (Reproduced with permission from ref 44)

ent dihydropyridines (Table 4). Although detection limits for nifedipine and nilvadipine were below 0.5 pg injected, S/N ratios for nicardipine and felodipine were roughly one order of magnitude lower and, in the case of felodipine, similar to what has been obtained with EI.

When isotope-labelled internal standards were used, detection of the analyte may be limited by spectral overlap, which in turn is influenced by the isotopic purity of the internal standard. For example, the concentration of [$^2H_3$]nilvadipine added to plasma had to be decreased from 1 to 0.1 ng/ml to lower the detection limit for nilvadipine from 0.10 to 0.01 ng/ml [43].

3.3. Liquid chromatography

The LC systems for separation of the dihydropyridines from other components in the extract from plasma generally consist of a C_{18} or C_8 bonded stationary phase and a mixture of phosphate or acetate buffer (pH 3–7) and methanol and/or acetonitrile as mobile phase. However, the mobile phase is not always buffered, since the analytes as a rule are non-protolytes. Separation of nifedipine on silica [34] or on diol phase [39] with a non-polar mobile phase is also used, in the latter case by direct injection of an aliquot of the organic extract. Oxidation to the corresponding pyridine compound as in early GC assays was employed by Sadanaga *et al.* [34]. Electrochemical detection at *ca.* +1.0 V potential has been used by two groups [16,36] both claiming 2 ng/ml as the detection limit. Monitoring by UV absorbance at 235 nm is generally chosen but measurements at 254, 280 and 355 nm are also possible The lower limit of quantification is 2 ng/ml, which is higher than that for GC methods but, at least for nifedipine, sufficient for therapeutic plasma levels.

Other dihydropyridines are generally separated and determined in the same manner as nifedipine. Nicardipine was separated on silica packing with a non-polar mobile phase [8] but that is an exception, and generally C_{18} packings and aqueous–organic mobile phases are used (Fig. 6). The limit of quantification is of the same order as that for nifedipine.

Like most other dihydropyridines, nivaldipine has a chiral carbon atom and is administered as a racemate. Tokuma *et al.* [44] separated the two enantiomers in a plasma extract on a Chiralpak OT(+) analytical column with methanol–water (95:5) as mobile phase. The two eluent fractions containing the nivaldipine enantiomers were collected, and the solvent was evaporated Each isomer was then determined by negative-ion GC–MS at a limit of detection of 0 025 ng/ml for each enantiomer. Triple-deuterated racemic internal standard was necessary throughout the analytical procedure (see Fig. 7).

3.4. Comparison of chromatographic techniques used for dihydropyridines

GC is very well suited for determination of dihydropyridines in biological

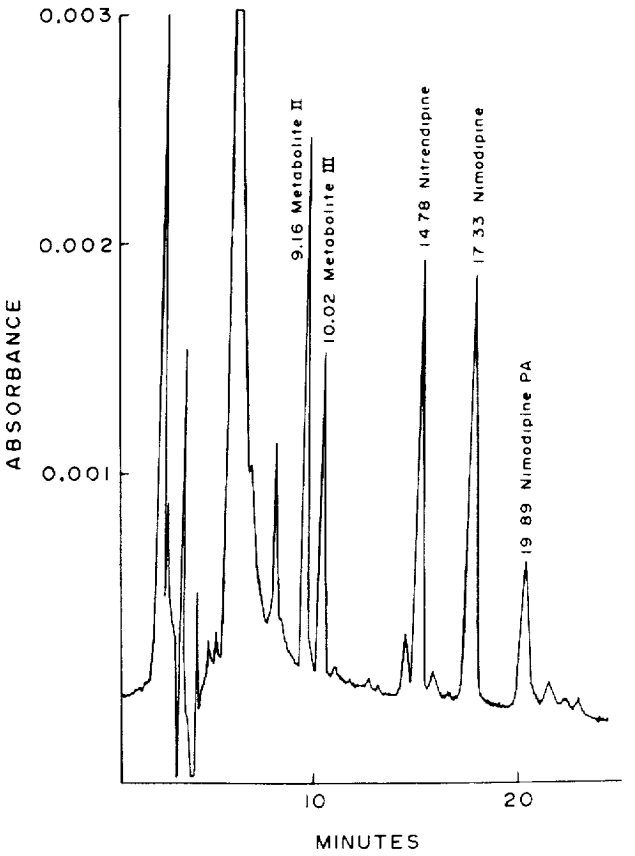


Fig. 6 LC separation of nimodipine, its pyridine analogue, two metabolites II and III and nitrendipine (internal standard) extracted from plasma, each present at 20 ng/ml Spherisorb ODS (3 μm) column was eluted with methanol–water (66 34, v/v) The detector was set at 238 nm (Reproduced with permission from ref. 36.)

fluids, and in many cases a simple extraction procedure followed by GC–ECD will be an adequate and efficient solution. Care has to be taken to keep oxidation of the dihydropyridine at a minimum. For more demanding tasks, GC–MS has to be considered, in many cases preferably with NICI. The sensitivity of assays based on LC has not always been sufficient, but as the performance of both the detectors and the separation columns improves, LC methods may become attractive alternatives to GC–ECD and GC–MS methods.

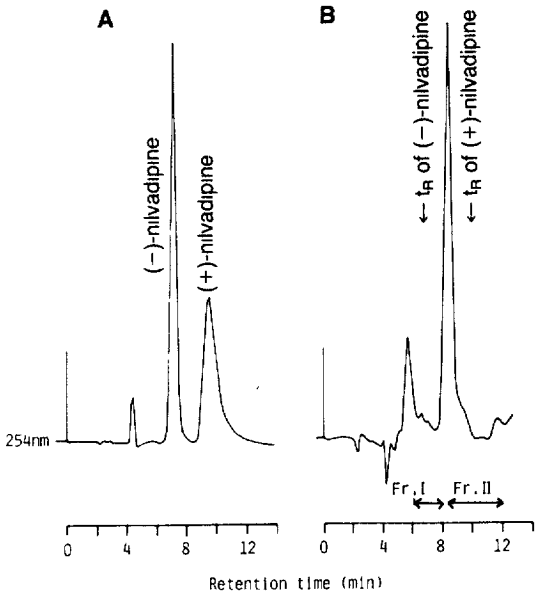
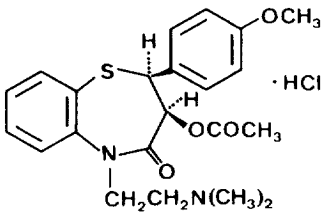


Fig 7 LC separation of (A) racemic nilvadipine and (B) extract from human plasma Column, Chiralpak OT(+) (25 cm × 4 6 mm I D), mobile phase, methanol–water (95·5, v/v). Fractions I and II were collected for GC–MS analysis (see Fig 5) (Reproduced with permission from ref 44)

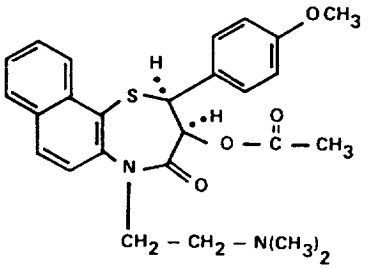
4 DILTIAZEM AND NALTIAZEM

4.1. Sample pretreatment

Diltiazem (Fig. 8) is a monovalent tertiary amine with a pK_a of 7.7 in its protonated form. The compound is isolated from plasma by liquid–liquid extraction at pH 7–9, over which range the yield is constant [56]. In most instances the extraction is performed without pH adjustment, *i.e.* at physiological pH. The reason why higher pHs are avoided is the increased risk for hydrolysis. Methyl *tert*-butyl ether is a popular organic solvent [57] and also hexane with 2% 2-propanol [58] or isopentanol [59], or hexane–diethyl ether [60]. The aqueous sample is extracted again with the same solvent [59] or with another solvent, dichloromethane, to promote extraction of metabolites [61]. Liquid–solid extraction [62] and protein precipitation with acetonitrile [63] have also been reported. Evaporation of the organic solvent precedes the GC assays and also LC. For the latter technique back-extraction into a small volume of diluted sulphuric, hydrochloric or phosphoric acid is often preferred, followed by reversed-phase LC Additional purification steps are in some instances included, particularly in GC methods [64,65].



Diltiazem



Naltiazem

Fig 8 Structures of diltiazem and naltiazem

4.2. Gas chromatography

GC methods for diltiazem are based on either nitrogen-selective or (with higher assay sensitivity) electron-capture detection. Sufficient sensitivity may be obtained without preconcentration of plasma extracts if small volumes of solvent are used [66]. Direct injection of plasma extracts requires, however, that the metabolite desacetyldiltiazem is adequately separated from the parent drug on the chromatographic column. Difficulties with this separation have led to procedures in which desacetyldiltiazem is trimethylsilylated, using bis(trimethylsilyl)-acetamide [64], N-methyl-N-trimethylsilylacetamide [67] or bis(trimethylsilyl)trifluoroacetamide [56]. Prior to injection of the sample, excess reagent is removed by evaporation. Since trimethylsilyl ethers generally have limited stability towards hydrolysis, it is uncertain whether this procedure can be used batchwise for automated GC.

Successful GC of diltiazem requires thoroughly deactivated columns. Packed columns of the phenylmethylsilicone type (OV-7 and OV-17) have been used, in one case with 20% phase loading (!). Proper column inertness is more easily obtained today with capillary columns than previously with packed columns and may be achieved also with less polar phases, such as 5% phenyl [56]. Typical elution temperatures are in the range 240–280°C.

Naltiazem (Fig. 8) is structurally closely related to diltiazem. One published method [68] employed extraction at pH 7.0 with *n*-hexane–ethylene dichloride–methyl *tert.*-butyl ether. After a concentration step, the extract was analysed by GC–MS with positive-ion chemical ionization. Methane was preferred to ammonia as reagent gas. The molecular ion MH^+ was detected, permitting plasma concentrations down to 2 ng/ml to be measured.

4.3. Liquid chromatography

LC separations of diltiazem have in most instances been performed with an octadecyl-bonded stationary phase. One of the first assays [57] used a cyano-bonded phase, and there are a few other instances of this kind of column being used. One recent paper described an assay that used a polymeric phase PRP-1 and a mobile phase of pH 10.5 [63]. A normal-phase system on silica and an organic mobile phase containing 0.3% ammonium hydroxide were suggested by Kinney and Kelly [60].

There may be at least six metabolites that appear in the chromatograms, of which the desacetyl and the N-demethyl ones are most frequently measured. There seems to be no real problem [69] of separating diltiazem from either its metabolites or endogenous compounds (see Fig. 9) Phosphate or acetate buffer solutions of pH 3–5, combined with organic modifiers, methanol or acetonitrile, are common components in the mobile phases used. Heptanesulphonate is added as ion-pairing agent [58,59,70] probably without much effect on retention or selectivity. This is even more valid in two other papers, with bromide [71] and tetrabutylammonium [63] as suggested ion-pairing additives.

The addition of an aliphatic amine, usually triethylamine, is a way to improve peak symmetry for diltiazem (a hydrophobic amine) and its metabolites. This approach has been adopted in a couple of papers. Höglund and Nilsson [69] also tested 1-dimethylaminododecane as a charged modifier to improve the chromatographic performance. The need for amine modifiers varies with the properties of the bonded stationary phase.

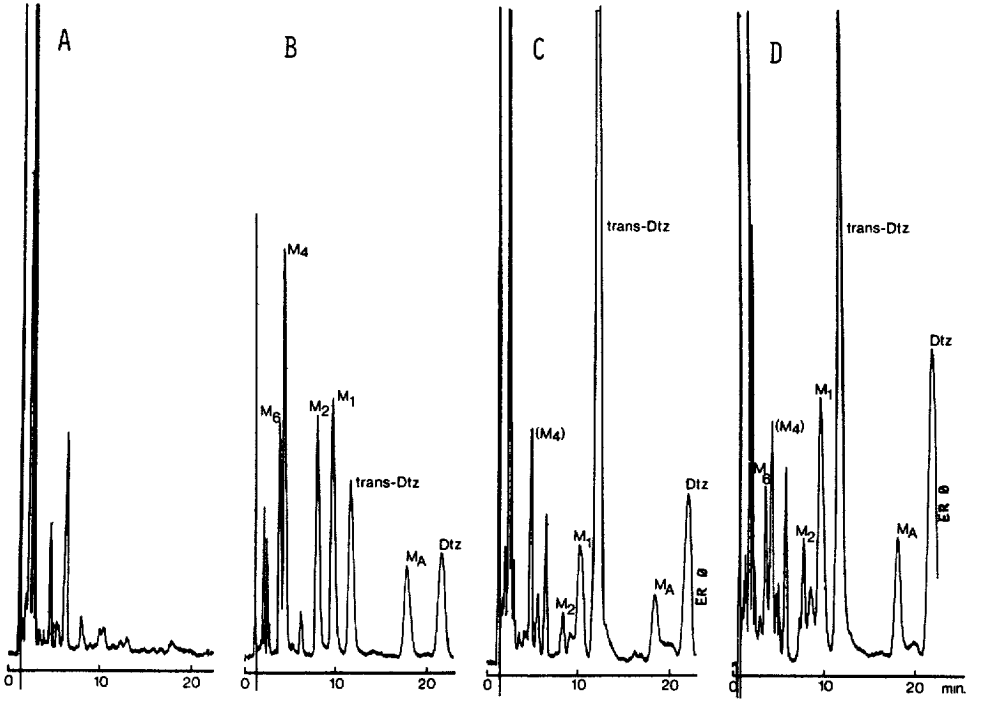


Fig 9 LC separation of diltiazem and metabolites extracted from human plasma. (A) Blank plasma, (B) blank plasma with diltiazem and metabolites added together with *trans*-diltiazem, (C) plasma from a subject 4 h after a single dose of 90 mg of diltiazem · HCl, diltiazem concentration 150 n*M* (70 ng/ml), (D) plasma from the same subject 4.5 h after dose during steady-state conditions (90 mg three times daily), M refers to metabolites Column, Novapak C_{18}, mobile phase, 25 m*M* phosphate buffer (pH 4.0)–acetonitrile (90·10) containing 0.6 m*M* 1-dimethylaminododecane; detection, UV at 237 nm (Reproduced with permission from ref. 69.)

Diltiazem in the eluent is monitored by UV detection, usually at *ca.* 237 nm, but 210 and 254 nm have also been used. Höglund and Nilsson [69] tested electrochemical detection, but a high working potential had to be applied, and it was not a viable alternative to UV detection in terms of sensitivity. The lower limit of quantitation reported is often *ca.* 5–10 ng/ml, although both lower and higher levels are given. The sensitivity of LC–UV seems sufficient to monitor therapeutic concentrations and for pharmacokinetic studies.

4.4. Comparison of chromatographic techniques used for diltiazem

Diltiazem has high detectability in the electron-capture detector and, with sufficiently inert capillary columns, GC is a well suited technique for the assay of low concentrations in plasma, as confirmed by our own experience. In some methods derivatization of the desacetyl metabolite is necessary to improve the separation from the parent drug.

LC is more frequently employed and seems to give adequate sensitivity, although not as high as GC–ECD. Despite UV detection at short wavelengths, the selectivity is sufficient owing to the hydrophobic character of the compound, which facilitates its separation from plasma components. Thus both LC and GC are useful techniques for the determination of diltiazem.

Table 5 summarizes the information on diltiazem and naltiazem

5 VERAPAMIL

5.1. Sample pretreatment

Verapamil (Fig. 10) is a tertiary amine of liphophilic character with a pK_a of 9.0 [77]; it is often determined together with a pharmacologically active metabolite, norverapamil, which is the N-demethylated form. Liquid–liquid extraction from plasma is most generally performed at strongly alkaline pH and with nonpolar organic solvents such as alkanes, pure [78,79] or with a few percent of butanol [80] or pentanol [81]. This approach is valid for GC methods, where re-extraction into an aqueous acidic phase and back-extraction into a new organic phase often precede the chromatographic assay. The purpose is to eliminate sample components that interfere with the chromatographic determination. Total recovery may still be quantitative [82], although several authors have chosen conditions that give lower recoveries. Norverapamil was extracted in lower yield and converted into the corresponding carbamate by derivatization with ethyl chloroformate, which improved both the extraction recovery and the GC properties of the analyte [82].

In LC methods diethyl ether is a popular solvent for verapamil [83,84] and back-extraction into a small-volume acidic aqueous phase seems to be the natural choice before reversed-phase LC [78,83]. In a few instances norverapamil is acetylated by addition of acetic anhydride, prior to back-extraction of verapamil, in order to avoid interference in the chromatogram [81,84]. Solid-phase extraction has been reported in one case [85] and so has protein precipitation by addition of methanol [86] or acetonitrile [87]. Extraction recovery is generally above 90%, but surprisingly low figures down to 60–70% [88,89] are also given without comment. In one study [90] an aliquot of the organic phase, methyl *tert.*-butyl ether, was injected directly into a normal-phase LC column.

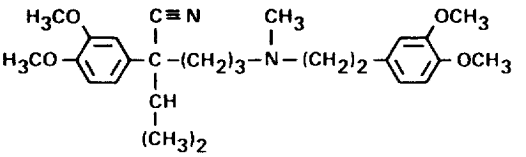


Fig 10 Structure of verapamil

TABLE 5

CHROMATOGRAPHIC METHODS FOR DILTIAZEM AND NALTIAZEM

GC or LC	Column	Detection	Pretreatment	LLQ (ng/ml)	Detection limit (ng/ml)	Remarks	Ref
Diltiazem							
GC	p, OV-17	NPD	2E+C+D+C	10		Trimethylsilyl derivative of desacetyldiltiazem	64
GC	p, OV-17	ECD	E	1			66
GC	p, OV-17	NPD	E+C		1	Lyophilized plasma	65
GC	p, OV-7	ECD	E+C+D+C	2		Trimethylsilyl derivative of desacetyldiltiazem	67
GC	c, DB-5	ECD	2E+C+D+C	3			56
LC	CN	UV 237 nm	E+B	10		Plus desacetyldiltiazem	57
LC	C_{18}	UV 210 nm	E+S+C	100		Urine samples, plus six metabolites	72
LC	C_{18}	UV 240 nm	E+C	5		Dog plasma, plus desacetyldiltiazem	58
LC	C_{18}	UV 254 nm	E+B	2.5		Plus desacetyldiltiazem	70
LC	C_{18}	UV 237 nm	E+B+C	1		Plus four metabolites	71
LC	Si	UV 240 nm	E+C	2		Plus desacetyldiltiazem	60
LC	C_{18}	UV 230 nm	2E+B	5		Plus two metabolites	59
LC	C_{18}	UV 238 nm	S	5	2	Plus three metabolites (1989) Column switching	62, 72a
LC	CN	UV 239 nm	E+C		2	Plus desacetyldiltiazem	73
LC	C_{18}	UV 237 nm	E+B	10		Plus five metabolites	69
LC	CN	UV 240 nm	E	20		Plus verapamil	74
LC	C_{18}	UV 237 nm	E+B	10		Plus four metabolites	75
LC	PRP-1	UV 254 nm	P		10		63
LC	C_{18}	UV 237 nm	2E+C	2.5		Plus six metabolites	61
LC	CN	UV 237 nm	E+B	10		Plus desacetyldiltiazem	76
Naltiazem							
GC	p, OV-1	MS, PICI	E+C	2			68

5.2. Gas chromatography

GC methods for verapamil are based on the use of either the thermoionic nitrogen–phosphorus detector, or a mass spectrometer. Both detection techniques offer the required sensitivity, but their selectivity appears somewhat limited. Sample pretreatment procedures are designed to avoid crude extracts containing components that may disturb the measurement of low concentrations of the analyte. This is done either by using back-extraction or by choosing a less efficient solvent for the extraction and accepting a lower recovery.

Verapamil is eluted from GC columns packed with standard phase at column temperatures of *ca.* 270–290°C. In order to keep separation times short, short columns have been used, with one notable exception. Hoffman and Higgins [85] employed a 60 m × 0.25 mm I.D. fused-silica column (film thickness 0.1 μm) to resolve verapamil and its heptadeutero analogue. Thereby, both compounds could be selectively determined without the use of a mass spectrometer. The *n*-propyl isomer of verapamil was used as internal standard for both analytes. In spite of the length of the column, separation was achieved in less than 25 min, using hydrogen as carrier gas.

Amines are prone to adsorption onto active surfaces, and when non-polar solvents such as *n*-alkanes are used care has to be taken to minimize losses. Rosseel and Belpaire [91] found it necessary to silanize glassware and to avoid complete evaporation of the solvent during concentration of extracts Hoffman and Higgins [85] employed a cleaning procedure with hot sodium hydroxide for the GC injector glass insert at regular intervals, to maintain proper sample recovery and chromatographic efficiency.

Mass-selective detection of verapamil and norverapamil has been carried out with electron-impact ionization. Verapamil is detected by monitoring the base fragment at *m/z* 303, arising from the elimination of the dimethoxybenzyl group from the molecular ion. When [^{13}C,^{2}H$_2$]verapamil was used as internal standard [92], it contributed significantly to the signal at *m/z* 303, and samples had to be analysed in triplicate with different amounts of internal standard. Andersson *et al.* [82] used [^{2}H$_3$]verapamil, apparently without such interference.

5.3. Liquid chromatography

Most LC separations of verapamil have been performed on a C_{18} or a C_8 bonded phase with a mobile phase of phosphate or acetate buffer at pH 2–6 In a few instances ion-pairing agents have been added, such as perchloric acid [93], propanesulphonic acid [83] and heptanesulphonate [94]. Verapamil is a hydrophobic amine, and a high content of organic modifier, most often acetonitrile or methanol, is needed for a suitable retention.

Other bonded stationary phases employed are phenyl [95], cyano [89], diol [88] and cation exchanger [96]. With cyano and diol phases a neutral aqueous buffer

has been used in the mobile phase. In one study [90] non-modified silica was the stationary phase and a methanolic solution of potassium bromide and perchloric acid the mobile phase. The extract from plasma in methyl *tert.*-butyl ether is then injected directly with an efficient separation as a result. The major and active metabolite norverapamil has often been assayed simultaneously [86,90,97], as have other metabolites [79,94,95]. Difficulties in separating verapamil from norverapamil led to the use of acetylation of the latter in the work-up procedure [81,84].

In a recent paper, chiral separation of the enantiomers of verapamil and norverapamil was described [98]. The extract from plasma was injected into a column with a shielded hydrophobic phase for separation of verapamil from norverapamil. The fractions were then selectively transferred to a Chiral-AGP column, in which the enantiomers were resolved and separately quantitated. Because of the lower column efficiency, the lower limit of the assay with the coupled-column system was 50 ng/ml in the sample, whereas 7 and 10 ng/ml were the values when only the intact racemates were measured. As in all other LC methods detection was achieved by the native fluorescence of the compounds. Excitation at low wavelengths, often *ca.* 203 nm, sometimes *ca.* 280 nm, and emission monitored at 320 nm give high detectability with a reported limit of quantitation down to 1 ng/ml (Fig. 11).

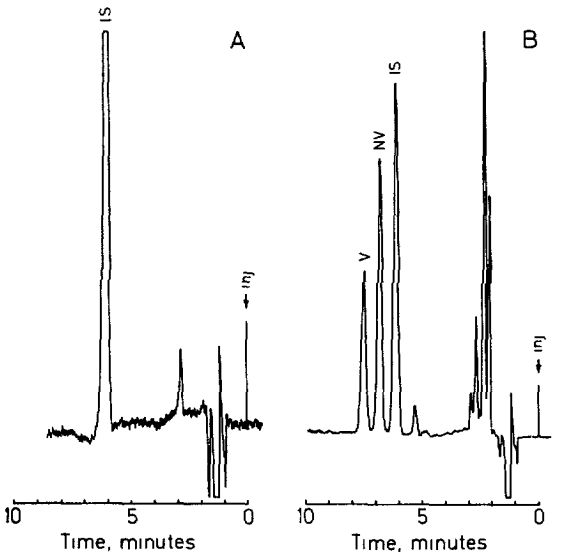


Fig 11 LC separation of verapamil (V), norverapamil (NV) and internal standard (IS) from a patient's plasma (right) and blank plasma (left) Column, Supelcosil LC-18 DB, 5 μm, mobile phase, 0 1 *M* phosphate buffer (pH 3.0)–acetonitrile (70·30, v/v) Fluorescence detection excitation 203 nm, emission >320 nm Peaks correspond to 46 ng/ml verapamil and 78 ng/ml norverapamil The blank was recorded at five times greater sensitivity (Reproduced with permission from ref 80)

5.4. Comparison of chromatographic techniques used for verapamil

Verapamil has a molecular mass of 454, and needs a high elution temperature and a short GC column for short separation times. The limited selectivity of the thermoionic nitrogen–phosphorus detector has been compensated for by improvements in the work-up procedure, which is less rigorous when mass-selective detection is used.

The inherent fluorescent properties of verapamil and norverapamil, and their hydrophobicity make LC a suitable technique for the measurement of low concentrations of these compounds in plasma. Table 6 summarizes the information on the chromatography of verapamil.

6 PIPERAZINE DERIVATIVES

These include cinnarizine, flunarizine and PU-122.

6.1. Sample pretreatment

Flunarizine and cinnarizine (Fig 12) are strongly hydrophobic piperazine derivatives with basic properties. Flunarizine has pK_{a1} = 7.71 and pK_{a2} = 4.40

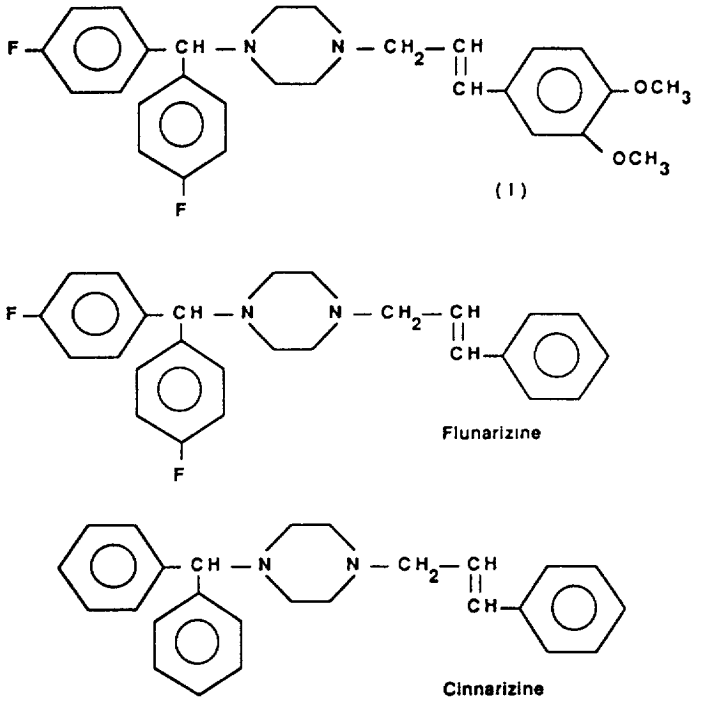


Fig. 12 Structures of PU-122 (I), flunarizine and cinnarizine

TABLE 6

CHROMATOGRAPHIC METHODS FOR VERAPAMIL

GC or LC	Column	Detection	Pretreatment	LLQ (ng/ml)	Detection limit (ng/ml)	Remarks	Ref
GC	p, Dexsil	MS, EI	2E + B + 2E + D + C	1			92
GC	p, OV-17	NPD	2E + B + 2E + C	4			99
GC	p, SP-2250	NPD	E + B + E + C		5		100
GC	c, SE-30, *m/z* 303	MS, EI	E + B + D + E + C	1		Ethyl carbamate of norverapamil	82
GC	p, SP-2250	NPD	E + B + E	0.5			101
GC	p, OV-1	NPD	E + C	10			102
GC	c, SE-52	NPD	S + C	5		GC separation of [$^2H_7$]verapamil	85
GC	c, 5% phenyl	NPD	E + C		1		91
LC	C_{18}	Flu 203/320 nm	E + B	1		Plus norverapamil (1980)	83, 97
LC	C_{18}	Flu 310/390 nm	E + D + B	5		Acetamide of norverapamil	84
LC	C_8	Flu 203/320 nm	E + B + E + C + D + C	10		Acetamide of norverapamil	81
LC	C_{18}	Flu 203/320 nm	2E + C	2 5		Plus seven metabolites	94
LC	Si	Flu ex 203 nm	E	2		Plus norverapamil	90
LC	C_{18}	Flu 203/320 nm	E + B	1			78
LC	SCX	Flu ex 203 nm	2E + B		5		96
LC	C_8	Flu 278/320 nm	P		5	Plus norverapamil	86
LC	Diol	Flu 280/314 nm	E + B + E + C		1	Plus norverapamil	88
LC	C_{18}	Flu 203/320 nm	E + B		4	Plus two metabolites	79
LC	C_{18}	Flu 214/>300 nm	P	40			87
LC	CN	Flu 201/320 nm	E + C		2	Plus norverapamil	89
LC	Phenyl	Flu ex 209 nm	E + C		1	Plus four metabolites	95
LC	C_{18}	Flu 203/320 nm	E + B	1		Plus norverapamil	80
LC	C_{18}	Flu 220/300 nm	E + B	1		Plus norverapamil	93
LC	Hisep + Chiral AGP	Flu ex 227	E + C	50		Plus norverapamil, chiral separation	98

[103]. Extraction from biological samples can be performed over a wide pH range, owing to the high distribution of the base to organic solvents, especially chlorinated hydrocarbons such as chloroform and dichloromethane. In fact, the distribution is too high to admit quantitative back-extraction from these solvents. Pentane–2-propanol gave quantitative recovery from buffered plasma (pH 8.5) after two consecutive extractions [104], as did dichloromethane after a single 30-min extraction under strongly alkaline conditions [105]. Nevertheless, quantitative recovery seems to be more readily obtained under moderately acidic conditions [106,107]. The extraction kinetics are probably more favourable at low pH, where the piperazines are more soluble in the aqueous phase.

Solvents that cause less protein denaturation are preferred. For example, chloroform–hexane (2:3) gave higher recovery of cinnarizine from plasma than chloroform alone, although both gave quantitative recovery from buffered (pH 3) aqueous solutions [107]. Flunarizine has been extracted from plasma with dichloromethane after addition of 1 *M* hydrochloric acid, giving a pH less than 2 [108]. In this case, formation of chloride ion pairs could possibly contribute to the extraction. Liquid–solid extraction at pH 4.5 with Sep-Pak C_{18} cartridges has been used to extract flunarizine from plasma with 92.7% recovery [106].

Back-extraction into an acidic aqueous phase has been used in combination with weakly polar organic solvents such as diethyl ether [109] or pentane–2-propanol [104]. Back-extraction of flunarizine may involve the risk of irreversible losses, according to Kapatenovic *et al.* [104]. They found it necessary to use silylated glassware and to minimize the contact time with the hydrochloric acid. With sulphuric acid, losses could not be avoided.

6.2. Gas and liquid chromatography

The piperazine moiety of flunarizine and cinnarizine favours GC with nitrogen-selective detection. High assay sensitivity, around or below 1 ng/ml, is obtained when combined with a sample pretreatment procedure that includes back-extraction. Proper care has to be taken to avoid adsorption losses during sample handling and chromatography. Extensive syringe cleaning was used between (automated) injections in order to eliminate sample carry-over [104].

The LC separation of flunarizine and cinnarizine in extracts from plasma samples is generally performed with a C_{18} bonded stationary phase and a weakly acidic or neutral mobile phase with a content of organic modifier as high as 90% methanol [107]. In a few instances a low-molecular-mass aliphatic amine has been added to the mobile phase to decrease peak tailing [106,108]. UV detection, often at *ca.* 250 nm, allows the determination of plasma levels down to 5 ng or less

6.3. Comparison of chromatographic techniques used for piperazine derivatives

An assay sensitivity (LLQ) of 1 ng/ml or lower can be obtained by GC with

TABLE 7

CHROMATOGRAPHIC METHODS FOR PIPERAZINE DERIVATIVES

GC or LC	Column	Detection	Pretreatment	LLQ (ng/ml)	Detection limit (ng/ml)	Remarks	Ref
Flunarizine							
GC	p, OV-17	NPD	2E+B+2E+C	0 5			110
GC	p, OV-17	NPD	2E+B+2E+C	1 5			111
1983							
GC	c, DB-1	NPD	2E+B+2E+C	0 25			114
GC	p, OV-17	NPD	S+C	1			112
LC	C_{18}	UV 254 nm	E+C	4			108
LC	Si	UV 255 nm	E+C	2			105
LC	C_8	UV 254 nm	2E+B+E+C	7			113
LC	C_{18}	UV 250 nm	S+C		13		106
LC	C_8	UV 210 nm	E+C+S+C	2			114
Cinnarizine							
GC	p, OV-17	NPD	2E+B+2E+C	0 5		Plus flunarizine	110
LC	C_8	UV 250 nm	E+C		2		115
LC	C_{18}	UV 254 nm	E+C		2		107
PU-122							
GC	c, DB-1	NPD	E+C	5			116

nitrogen-selective detection, but this has been difficult to achieve with LC. GC of these piperazine derivatives is, however, not straight-forward owing to adsorption phenomena in the GC system and in solvents used in GC for injection. Back-extraction procedures have to be designed with care to avoid losses of the analyte, and it will be an advantage if sufficient selectivity is obtained without back-extraction.

Table 7 summarizes the information on the chromatography of flunarizine, cinnarizine and PU-122.

7 PRENYLAMINE

Published methods for prenylamine (Fig. 13) are few. GC with flame-ionization detection has been used with moderate sensitivity [117]. Prenylamine was isolated from plasma or urine by extraction into *n*-heptane and back-extraction into 1 *M* acetic acid. Prenylamine, underivatized or as the acetyl or propionyl derivative, was separated from potential metabolites on a packed column, the detection limit being 50 ng/ml. Higher sensitivity would be expected with nitrogen-selective or mass-selective detection. LC detectors offer poor sensitivity for prenylamine, but detectability may be markedly increased by derivatization. *R*-(−)-Naphthylethyl isocyanate has been used as a chiral fluorescence marker [118], permitting separation and fluorescence detection of (*R*)- and (*S*)-prenylamine as their diastereomeric naphthylethylurea derivatives. When this technique was applied to plasma samples, from which the prenylamine enantiomers were isolated by extraction, back-extraction and reextraction, the detection limit was below 1 ng/ml.

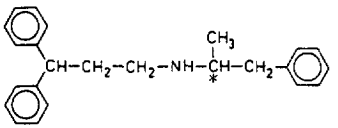


Fig 13. Structure of prenylamine.

8 CONCLUDING REMARKS

GC and LC instrumentation has become very sophisticated. However, the development of column technology in GC and in reversed-phase LC appears to have levelled off. Although electron-capture and nitrogen-selective detectors for GC have not changed much over the past ten years, UV and fluorescence detectors for LC are still being improved. The progress of MS detectors makes it easier to exploit this powerful technique for routine work in an analytical laboratory.

An increase in plasma assay selectivity and sensitivity for drugs, such as calcium channel blockers, may be achieved by increased human effort or improved

instrumentation resource. Since these always are limited, methods that are too complicated or employ exclusive instrumentation will not be widely used. For calcium channel blockers, existing analytical methods for their determination in plasma exhibit a sensitivity and selectivity that are usually sufficient for present demands. However, one can expect that GC and LC procedures with improved sensitivity and with additional selectivity will be required, *e.g.* for dihydropyridines in lower therapeutic doses and for enantioselective determinations.

9 SUMMARY

Numerous publications during the past ten years have described the determination of various calcium channel blockers in biological fluids, using gas and liquid chromatographic techniques Diltiazem, verapamil, flunarizine and a growing number of dihydropyridines belong to this group of drugs, which in most instances are active at low plasma concentrations. From a bioanalytical point of view these compounds have many features in common, such as high lipophilicity and favourable detection properties.

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