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Analysis of nifedipine and its pyridine metabolite dehydronifedipine in blood and plasma: review and improved high-performance liquid chromatographic methodology

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Abstract: A reversed-phase HPLC method is described for the simultaneous determination of nifedipine and its primary pyridine metabolite dehydronifedipine in blood and plasma, that involves UV detection and neutral (blood) or alkaline (plasma) extraction. The limit of reliable determination is found to be 3 ng ml^{-1} with an inter-assay RSD of below 11%. In the presence of haemoglobin, nifedipine is unstable at pH > 10, necessitating neutral extraction for the measurement of nifedipine in haemolysed blood. Published methods for analysis of nifedipine are reviewed, emphasizing the lack of specificity and sensitivity which render many of them unsuitable for the investigation of nifedipine disposition in man.

Keywords: *Nifedipine; dehydronifedipine; plasma; blood; review.*

Introduction

Nifedipine (**I**; Fig. 1), the archetype of the dihydropyridine calcium entry blockers, is used widely in the treatment of hypertension and angina pectoris, and also is increasingly used as probe drug to assess cytochrome P-450 IIIA4 enzyme activity *in vivo* [1]. In man nifedipine is metabolized predominantly by oxidative dehydrogenation to dehydronifedipine (**II**; Fig. 1), which in turn is further metabolized to more polar compounds [2]. Measurement of dehydronifedipine is important for mechanistic interpretation of disposition studies with nifedipine [1], or when dehydronifedipine itself is used as a probe drug in experimental animals [3].

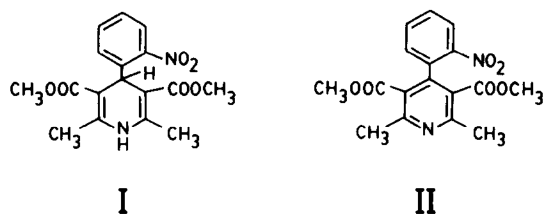

Figure 1
Structures of nifedipine (**I**) and dehydronifedipine (**II**).

Nifedipine is highly sensitive to light [4–11] and chemical oxidation [12–14], upon which dehydronifedipine and the nitroso-analogue of dehydronifedipine can be formed. During exposure to sodium light only, decomposition of nifedipine cannot be detected within the time-frame of analysis (several hours). Thermo-oxidation of nifedipine [12, 14, 15], its low plasma levels in man (1–200 ng ml^{-1}) and its extensive metabolism have complicated the development of specific analytical methods.

Published methodology

Approximately 50 methods for the determination of nifedipine concentrations in biological fluids, applying a variety of analytical techniques, have been published up to 1990 [2, 4–6, 9–53]. The major characteristics of these assays are summarized in Tables 1–4. However, almost half of them are not useful for studying nifedipine disposition in man because of their lack of sensitivity (⩾10 ng ml^{-1}) or specificity, or because their applicability to biological material has not been proven. A major cause for non-specificity is the oxidation of nifedipine to dehydronifedipine, either deliberately prior to chromatography [12–14, 16, 17], or because of thermo-oxidation in the

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Table 1
Major characteristics of packed-column GC assays of nifedipine

Ref.	Extraction*	Internal standard†	Stationary phase	Mobile phase*	Detection‡	Nifedipine-specific?§	Measures dehydronifedipine?	Limit of assay§
[5]	Toluene	–	OV-101	Nitrogen	ECD	Yes	No	1 (8)
[10]	Benzene	–	OV-101	Nitrogen	FID	Yes	No	300 (8)
[12]	Benzene	+	OV-1	Helium	MSD	No (5)	No	5 (8)
[13]	Benzene	+	OV-1	Nitrogen	ECD	No (5)	No	5 (7)
[14]	die	+	OV-101	arg–meth	ECD	No (5)	No	?
[15]	cyclo–etac	–	OV-17	arg–meth	ECD	No (6)	No	10 (8)
[16]	Benzene	+	OV-17	Nitrogen	ECD	No (5)	No	2 (7)
[23]	etac	None	OV-17	Nitrogen	radio	Yes	No	5 (7)
[26]	Toluene	–	OV-17	arg–meth	ECD	Yes	Yes	1 (7)
[27]	Toluene	–	OV-17	Nitrogen	ECD	Yes	No	1 (7)
[28]	dee	–	OV-17	arg–meth	ECD	Yes	No	1 (8)

*die, Di-isopropylether; cyclo, cyclohexane; etac, ethylacetate; dee, diethylether; arg, argon; meth, methane.
†+, (Dihydro)pyridine-analogue; –, not related to nifedipine.
‡radio, Radioactivity (^{14}C or ^{3}H).
§(5) Pre-column oxidation to dehydronifedipine; (6) on-column oxidation to dehydronifedipine; (7) detection limit (ng ml^{-1}); (8) limit of reliable determination (ng ml^{-1}).

Table 2
Major characteristics capillary-column GC assays of nifedipine

Ref.	Extraction*	Internal standard†	Stationary phase‡	Mobile phase‡	Detection	Nifedipine-specific?‡	Measures dehydronifedine?	Limit of assay‡
[2]	Toluene	+	DB-1 (4)	Helium (1)	ECD	Yes	Yes	1 (8)
[11]	Toluene	+	SE-30 (4)	Helium (1)	ECD	Yes	No	0.5 (7)
[29]	Benzene	–	OV-1 (3)	Helium (1)	ECD	Yes	No	1 (7)
[29]	Benzene	–	OV-1 (3)	Helium (1)	FID	Yes	No	20 (7)
[30]	Toluene	None	DB-1 (4)	Helium (1)	ECD	Yes	No	2 (8)
[31]	Toluene	+	DB-5 (4)	Hydrogen (1)	ECD	Yes	No	2 (8)
[32]	Toluene	+	DB-1 (4)	Hydrogen (1)	ECD	Yes	No	1 (8)
[33]	iso–Toluene	+	DB-1 (4)	Helium	MSD	Yes	No	2 (8)
[34]	Toluene	+	DB-1 (4)	Helium (1)	ECD	Yes	No	1 (7)
[35]	Toluene	+	OV-1 (4)	Helium	NPD	Yes	No	0.5 (7)
[36]	Toluene	+	OV-1 (3)	Helium (1)	ECD	Yes	Yes	1 (7)
[37]	Toluene	–	SE-30 (4)	Helium	ECD	Yes	No	2 (7)
[38]	Benzene–hex	None	HP-5 (4)	Helium (1)	ECD	Yes (9)	No	0.01 (7)

*iso, Isoamyl alcohol; hex, hexane.
†+, (Dihydro)pyridine-analogue; –, not related to nifedipine.
‡(1) Temperature programmed; (3) glass capillary column; (4) fused-silica column; (7) detection limit (ng ml^{-1}); (8) limit of reliable determination (ng ml^{-1}); (9) not applied to biological material.

Table 3
Major characteristics of HPLC assays (reversed-phase and normal-phase) of nifedipine

Ref.	Extraction*	Internal standard†	Stationary phase‡	Mobile phase*	Detection (nm)‖	Nifedipine-specific?§	Measures dehydronifedipine?	Limit of assay§
[6]	dcm–Pentane	+	C18	acn–buf 4.0	238	Yes	No	2 (8)
[9]	etac	–	C18	meoh–buf 6.1	254	Yes	No	10 (7)
[14]	dee	+	C18	meoh–Water	254	Yes	No	?
[26]	Toluene	–	C18	acn–meoh–Water	235	Yes (9)	No	?
[29]	Benzene	–	C18	acn–Water	235	Yes	No	5 (7)
[39]	Solid-phase	–	C8	acn–buf 3.0	236	Yes	No	10 (8)
[40]	Solid-phase	–	C18	meoh–buf 6.1	235	Yes	No	3 (8)
[41]	dee–Hexane	+	C18	meoh–Water	236	Yes	No	2 (7)
[42]	chlf	–	C18	acn–meoh–buf 6.1	235	Yes	No	5 (7)
[43]	acn	–	C18	meoh–buf	280	Yes	No	5 (8)
[44]	Solid-phase	None	C18	acn–buf 4.0 (2)	355	Yes	No	0.5 (7)
[45]	dcm–Pentane	–	C8	acn–buf 3.2	238	Yes	No	3 (7)
[46]	dee	+	C8	meoh–buf 4.0	350	Yes	No	10 (7)
[47]	etac	+	C8	meoh–Water	254	Yes	Yes	10 (7)
[48]	Solid-phase	+	C8	meoh–Water	235	Yes	No	5 (7)
[49]	Toluene	+	C18	acn–buf 3.5 (2)	229	Yes	Yes	3 (8)
[52]	etac–hexane	+	C18	acn–meoh–buf 7.0	ec	Yes	No	2 (8)
[53]	Toluene	+	C18	meoh–thf–buf 3.0	ec	Yes	No	2 (7)
[17]	chlf	–	diol	hex	280	No (5)	No	2 (8)
[50]	chlf–Hexane	None	Si	acn–hex–ipa	235	Yes	No	1 (7)
[51]	dcm	–	Si	amm–dcm–form–hex–thf	237	Yes (9)	No	8 (7)

* dcm, Dichloromethane; etac, ethylacetate; dee, diethylether; chlf, chloroform; acn, acetonitrile; buf, buffer pH; meoh, methanol; thf, tetrahydrofuran; hex, hexane; ipa, isopropanol; amm, ammonia; form, formamide.

† +, (Dihydro)pyridine-analogue; –, not related to nifedipine.

‡ C8, *Octylsilica stationary phase*; *C18, octadecylsilica stationary phase*; *Si, silica stationary phase*; *diol, diol stationary phase* (*Nucleosil-OH*).

§ (2) Gradient elution or column switching; (5) pre-column oxidation to dehydronifedipine; (7) detection limit (ng ml^{-1}) (8) limit of reliable determination (ng ml^{-1}); (9) not applied to biological material.

‖ ec, Electrochemical detection.

Table 4
Major characteristics of other assays of nifedipine

Ref.	Type of assay	Extraction*†	Stationary phase†‡	Mobile phase*†	Detection§	Nifedipine-specific?‖	Measures dehydronifedipine?	Limit of assay†‖
[4]	TLC	n.a.	Si	chlf–cyclo	colour	Yes	No	?
[21]	fluorometric	etac	n.a.	n.a.	fluor	No	No	300 (7)
[22]	radioactivity	n.a.	n.a.	n.a.	radio	No	No	?
[24]	RRA	n.a.	n.a.	n.a.	radio	No	No	4 (8)
[25]	RIA	n.a.	n.a.	n.a.	radio	No	No	0.1 (7)

*etac, Ethylacetate; chlf, chloroform; cyclo, cyclohexane.
†n.a., Not applicable; ?, not known
‡Si, Silica stationary phase.
§colour, Colour reaction; fluor, fluorescence; radio, radioactivity (^{14}C or ^{3}H).
‖(7) Detection limit (ng ml^{-1}); (8) limit of reliable determination (ng ml^{-1}).

gas chromatographic (GC) injection system [12, 14, 15]. The impact of this type of non-specificity on the interpretation of *in vivo* experimental data is not always appreciated [18–20, 54–59].

The early fluorometric [21] and thin-layer chromatographic methods [4] cannot be used for quantitative analysis in biological fluids because of lack of sensitivity and specificity. Methods based on the determination of radioactivity after administration of radiolabelled nifedipine [22] also are not specific, unless chromatographic separation is performed prior to measurement of radioactivity [23].

A radio receptor assay (RRA) [24] and a radio immunoassay (RIA) [25], although sensitive down to the low ng ml^{-1} range, find only limited application because of lack of specificity. Owing to this lack of specificity, several RRAs for measurement of other dihydropyridines could be applied for measurement of nifedipine as well [60–62]. RRAs do not detect inactive metabolites that lack the dihydropyridine structure, whereas cross-reactivity with structural analogues is an intrinsic problem of both methods. It seems possible, however, to raise polyclonal antibodies with a better specificity [63]. Production of monoclonal antibodies against dihydropyridines, necessary for long-term reproducibility, have not yet been reported. Evidence for the usefulness of the binding assays for studying the pharmacokinetics of dihydropyridines is rather limited [24, 60, 62].

Several GC methods using packed columns have been published [5, 10, 12–16, 23, 26–28]. Half of these packed GC methods, however, are not specific for nifedipine since they are based on oxidation of nifedipine to its pyridine metabolite prior to chromatography [12–14, 16] or because thermal oxidation of nifedipine has been shown to occur [12, 14, 15]. Using electron-capture detection (ECD), the limit of determination is approximately 1–2 ng ml^{-1}. Flame ionization detection (FID) [10] and mass spectrometric detection (MSD) [12] seem to be somewhat less sensitive.

GC methods employing capillary columns [2, 11, 29–38], most of them involving ECD, usually avoid thermal oxidation, and thus appear to be selective for nifedipine. The resolution on capillary columns is generally much better than on packed columns. FID [29] is less sensitive than nitrogen–phosphorus detection (NPD) [35] or ECD, which can measure down to approximately 0.5–1 ng ml^{-1}. The reported sensitivity of MSD, requiring much more complicated and expensive hardware, varies considerably: values from 0.01 ng ml^{-1} [38] up to 2 ng ml^{-1} [33] have been reported.

A large number of reversed-phase HPLC [6, 9, 14, 26, 29, 39–49] and normal-phase HPLC [17, 50, 51] assays applying UV detection, have been published. These assays differ in their extraction procedure, sample pretreatment and chromatographic conditions. The limit of determination of most of these assays is in the range of 2–5 ng ml^{-1}. Some of them [26, 51] have not been used for analysis of nifedipine in biological fluids in this concentration range up to now, or are not specific for nifedipine [17]. HPLC methods using electrochemical detection [52, 53] have a comparable limit of determination, but suffer from lack of robustness and are not applicable to the measurement of the (electrochemically inactive) metabolites of nifedipine.

In contrast to the large number of assays for nifedipine in plasma, only a small number of assays for dehydronifedipine have been published. Two authors reported assays for dehydronifedipine using HPLC [47, 49], whereas three GC assays have been reported [2, 26, 36]. In principle, however, several other chromatographic systems should be suitable for measurement of dehydronifedipine [64].

This paper reviews published methodology for the analysis of nifedipine and presents a simple reversed-phase HPLC method for selective, sensitive and simultaneous determination of nifedipine and dehydronifedipine in various biological fluids. Preliminary data on the stability of nifedipine and dehydronifedipine towards hydrolysis and oxidation are presented.

Experimental

Chemicals and chromatographic system

Nifedipine (**I**), dehydronifedipine (**II**) and nitrendipine (internal standard) were kindly supplied by Bayer AG (Wuppertal, Germany). Solutions of these substances in methanol were prepared and were stored at 0°C, excluding all light, without significant degradation, for at least 2 months. All other chemicals were of analytical grade (Baker, Deventer, The Netherlands), and organic solvents were freshly distilled prior to use. All glassware was

rinsed with methanol prior to use and dried at 250°C for 2 h.

The HPLC system consisted of a M-510 pump and WISP-712B autoinjector (both: Millipore-Waters, Milford, MA, USA), a Spectroflow® 757 absorbance detector operated at 230 nm (Applied Biosystems, Ramsey, NJ, USA), a Microspher® C18 100 × 4.6 mm i.d. cartridge column (Chrompack, Middelburg, The Netherlands) and a 20 × 2 mm i.d. guard column packed with Perisorb® RP-18 (Upchurch Scientific, Oak Harbor, WA, USA). Detector output was processed by means of a C-R3A reporting integrator (Shimadzu, Kyoto, Japan) in peak height mode. The mobile phase consisted of methanol–sodium acetate (pH 4.0; 0.1 M 6:4, v/v), and a flow rate of 0.5 ml min^{-1} was used.

Sample preparation and calibration

To 1.00 ml of plasma in a centrifuge tube, 25 μl 4 M sodium hydroxide was added. After mixing and equilibrating for 5 min, 4.0 ml of *n*-pentane–dichloromethane (7:3, v/v) and 100 ng internal standard (2 μg ml^{-1} in methanol) were added, and the samples were extracted for 20 min on a CM-9 multi-tube whirl-mixer (Sarstedt, Numbrecht, Germany). After centrifugation the upper organic layer was transferred to a clean centrifuge tube with Pasteur disposable pipettes and evaporated to dryness in 25 min using a vortex vacuum evaporator (Buchler, Fort Lee, NK, USA) at 30°C. The residue was dissolved in 75 μl mobile phase and transferred to clean glass inserts in black sample-vials. A volume of 20 μl was injected into the HPLC. The entire sample preparation procedure was performed in a room illuminated with only sodium light (SOX-E-18, Phillips, Eindhoven, The Netherlands), excluding all other light. For determination of nifedipine in blood, haemolysed with a 5-fold excess of water, 100 μl phosphate buffer (pH 7.0; 0.25 M) was used instead of sodium hydroxide.

For each assay a six-point calibration curve, prepared by spiking control plasma or blood with nifedipine and dehydronifedipine (final concentration 0–100 ng ml^{-1}), was processed identically and simultaneously. The ratio of the peak height of nifedipine or dehydronifedipine to the internal standard was calculated and calibration curves were constructed by linear regression analysis. Results of four assay validation procedures, performed on various occasions over 3 years, are summarized in terms of mean and relative standard deviation (RSD).

Investigation of stability

In pilot experiments the stability and degradation of nifedipine and dehydronifedipine under various assay conditions were investigated by incubating these compounds (0.5 μg ml^{-1} = 1.4 μM) in 1.0 ml of water or plasma at 20 and 37°C for 3 h, excluding all light. The pH was adjusted to the desired value with 4 M hydrochloric acid (pH 1) or 4 M sodium hydroxide (pH 12–13). Intermediate pHs were obtained by addition of 100 μl of sodium acetate (pH 4.0; 0.25 M), phosphate buffer (pH 7.0; 0.25 M) or carbonate buffer (pH 10.0; 0.25 M). The concentration of haemoglobin was varied by addition of small volumes of haemolysed human erythrocytes with known haemoglobin concentration. At regular time intervals the concentrations of nifedipine and dehydronifedipine were assayed as described for haemolysed blood, and the product of ester cleavage of dehydronifedipine, the carboxylic acid derivative of dehydronifedipine [1–2, 6] was detected (qualitatively only) according to ref. 65. Degradation kinetics were not determined in detail, but in most cases could be described satisfactorily by a mono-exponential function. As an indication of degradation rates the initial half-life ($t_{½}$) was calculated by least-squares regression analysis on logarithmic transformed data.

Results and Discussion

HPLC assay

The present assay, measuring nifedipine and dehydronifedipine simultaneously and specifically in both plasma and haemolysed blood, is simple, robust and easy to automate. Typical chromatograms obtained with human plasma samples are shown in Fig. 2. Some endogenous compounds seem to be co-extracted, but none interfere significantly with the detection of nifedipine or dehydronifedipine. Retention times of nifedipine, dehydronifedipine and internal standard were 6.8, 4.9 and 13.9 min respectively. Retention times fluctuated to some extent with varying room temperature; although this could be eliminated by control of the temperature of the system (at 30°C), it was not considered to be necessary for routine analysis. The assay was linear up to at least

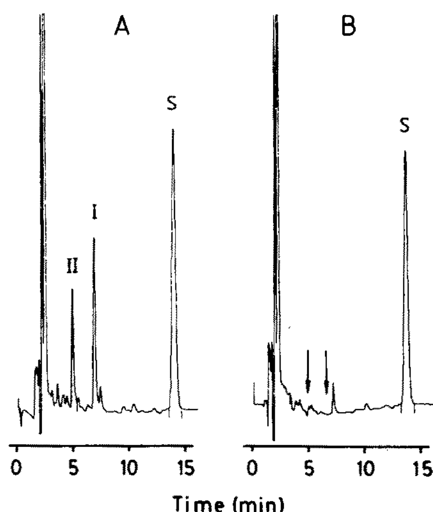

Figure 2
Chromatograms of a human plasma sample (A) containing 36.8 ng ml^{-1} nifedipine (**I**), 28.0 ng ml^{-1} dehydronifedipine (**II**) and 100 ng ml^{-1} internal standard (S), and of the corresponding blank sample (B).

500 ng ml^{-1} plasma for both compounds. Higher concentrations were measured after dilution of the samples. Calibration curves were always linear (nifedipine, $y = 0.0166x$; dehydronifedipine, $y = 0.0146x$; correlation coefficient always >0.995), and intercepts were only occasionally displaced from zero. The extraction ratio of all compounds was better than 90% with a relative standard deviation (RSD) below 4%. Precision, determined at 10 and 100 ng ml^{-1}, was always within 5% of the theoretical value, and the intra- and inter-assay RSDs, determined at 10 ng ml^{-1} (100 ng ml^{-1}) were below 8% (3%) and 11% (6%), respectively (Table 5). Although nifedipine and dehydronifedipine could be detected down to at least 1 ng ml^{-1}, the limit of reliable determination was arbitrarily set at 3 ng ml^{-1} plasma. The relative sensitivity of the assay for the two compounds could be tuned to some extent by changing the detection wavelength either towards the absorption maximum of nifedipine (238 nm) or dehydronifedipine (213 nm).

Occasionally, this assay also has been performed successfully with different apparatus, including other C18 analytical columns. In about 5 years over 8000 samples from a dozen of studies investigating the disposition and metabolism of nifedipine and dehydronifedipine in man and animals have been measured successfully with this assay.

Stability

A high pH (*ca* 12) for extraction from plasma was chosen in order to minimize the co-extraction of interfering compounds. For the assay from blood or from protein-free aqueous solutions a lower pH for extraction had to be chosen because of stability problems.

In protein-free aqueous solutions dehydronifedipine was hydrolysed quite rapidly to its carboxylic acid derivative at pH > 10. At pH 13 this hydrolysis apparently followed first-order kinetics with a $t_{1/2}$ of 0.3 h. In plasma the hydrolysis of dehydronifedipine appeared to be less rapid with a $t_{1/2}$ of at least 10 h at pH 12. Most likely the high protein binding of dehydronifedipine (95–97% P.A. Soons, unpublished results) protects dehydronifedipine from hydrolysis within the 3-h time-frame of our experiments. Nifedipine is more stable against base-catalysed hydrolysis in protein-free aqueous solution, its $t_{1/2}$ at pH 13 being approximately 2.6 h. The product formed was not identified. In plasma no degradation of nifedipine was detected at pH 12. At pH 1 in both protein-free aqueous solutions and in plasma nifedipine was slowly ($t_{1/2}$ > 20 h) degraded and the products formed were not identified. Under these conditions no degradation of dehydronifedipine was detected.

Table 5
Results of validation procedures for the assay of nifedipine and dehydronifedipine in human plasma. Values reported (mean ± SD) are the worst out of four validation procedures (n = 5–8 on each occasion)

	Nifedipine (%)		Dehydronifedipine (%)		Internal standard (%)
	10 ng ml^{-1}	100 ng ml^{-1}	10 ng ml^{-1}	100 ng ml^{-1}	200 ng ml^{-1}
Extraction ratio	>90 ± 4		>93 ± 4		>94 ± 3
Precision*	<5	<4	<5	<3	
Intra-assay RSD	<7	<3	<8	<3	
Inter-assay RSD†	<9	<6	<11	<6	

* Deviation from theoretical value.
† Total variability between assays.

An interesting observation was that both nifedipine and nitrendipine (the internal standard) were degraded in the presence of haemolysed erythrocytes at pH > 10 and pH 1, most likely by oxidation to their pyridine metabolites. At pH 1 indeed dehydronifedipine was identified as the major product formed from nifedipine. At pH > 10 dehydronifedipine could not be identified as the product formed because of its own instability under these conditions. The mechanism of this oxidation is unclear at present but it can be speculated that haemoglobin, present at a 10–100-fold excess in the incubations, was responsible for the oxidation. The rate of oxidation was positively related to the haemoglobin concentration and to temperature. At an initial haemoglobin concentration of 64 μM the apparent $t_{1/2}$ of nifedipine in plasma at pH 12 was 1.6 h. In water at pH 13 an apparent $t_{1/2}$ of 1.4 h was already obtained with an initial haemoglobin concentration of 15 μM. In water at pH 1 an apparent $t_{1/2}$ of 4.4 h was obtained with an haemoglobin concentration of 150 μM, and the loss of nifedipine could be completely accounted for by the appearance of dehydronifedipine. At pH 3, 7 and 10 no degradation of neither nifedipine nor dehydronifedipine was observed in plasma, haemolysed blood and protein-free aqueous solutions. Photooxidation of nifedipine was also reported to be pH-dependent, with its optimal stability at pH 5 [8]. The oxidation reaction could be imitated with an excess (100 μM) of ferric-ions or cericions at pH 1. The observed oxidation of nifedipine and nitrendipine in the presence of haemoglobin at low and high pH needs further investigation of its mechanisms and its *in vivo* relevance.

Choice of method

A variety of methods have been published for the quantitative analysis of nifedipine [2, 4–6, 9–53]. Many of them, however, lack the necessary specificity [12–17, 21, 22, 24, 25] or sensitivity [4, 9, 10, 15, 21, 29, 39, 46] to be used in single-dose nifedipine disposition studies in man. Only a few methods have been applied to the simultaneous analysis of nifedipine and dehydronifedipine, which allows a more detailed mechanistic interpretation of kinetic/metabolic studies with nifedipine. Of those assays, one published HPLC assay [49] and the present one are more easy to automate and can be implemented more easily in most laboratories than the GC-based assays [2, 26, 36]. The sensitivity of these assays is comparable, but capillary-GC assays [2, 36] may have the advantage of a better resolution. The sensitivity of one published HPLC assay for simultaneous measurement of nifedipine and dehydronifedipine is too low for use in single-dose disposition studies [47].

The low boiling points of dichloromethane (40°C) and pentane (36°C), a mixture of which is used for extraction, allows concentration of the extract under very mild conditions, especially compared with toluene (b.p. 110°C) which has been used in many assays. The use of benzene should be regarded a major disadvantage because of its well known carcinogenic properties. Ideally, chromatographic analysis of nifedipine should incorporate an internal standard structurally related to nifedipine, possessing similar chemical and physical properties [66]. The use of an internal standard that is not structurally related to nifedipine [5, 9, 10, 15, 17, 26–29, 37, 39, 40, 42, 43, 45, 51] or of no internal standard at all [23, 30, 38, 44, 50] is certainly not optimal.

Several authors have applied extraction under strongly alkaline conditions. As indicated by our pilot experiments, erroneous results are to be expected if these assays are applied to haemolysed blood or haemolytic plasma. In those cases the pH of extraction should be below 10. Because of the high sensitivity of nifedipine to normal laboratory light [4–11] all sample handling must be performed in a completely darkened room illuminated only with sodium light.

Conclusion

Several useful methods for the measurement of nifedipine in biological fluids are currently available, and also a few assays for its pyridine metabolite. Only methods selective towards nifedipine should be considered for use, whereas the ultimate choice may depend on the type of samples, the number of samples, sensitivity and resolution needed, and available analytical facilities. Pharmacokinetic data and concentration–effect relationships for nifedipine, obtained with non-specific assays should be ignored. Most of the considerations on the analysis of nifedipine apply equally well to other dihydropyridine calcium entry blockers.

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