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Note

Study of nifedipine photodecomposition in plasma and whole blood using capillary gas—liquid chromatography

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Nifedipine [dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine carboxylate] is a widely used antihypertensive and antianginal drug belonging to the group of compounds known as a calcium channel antagonists. It exerts its effect by blocking calcium channels and consequently, retarding the passage of calcium ions across cell membranes. This causes vasodilation and lowering of peripheral resistance.

Nifedipine analysis is usually performed using gas—liquid chromatography (GLC), which is more sensitive than high-performance liquid chromatography (HPLC) [1, 2]. Previously reported GLC assays for nifedipine have employed a packed column [3—5] but the use of a fused-silica capillary column [6] confers improved peak shapes and greater sensitivity over those obtained on a packed column. We have used such a capillary GLC method in this study.

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Nifedipine is very light sensitive, spontaneously forming its nitroso derivative on exposure to daylight [3, 4] and nitropyridine derivative on exposure to UV light [4]. Some investigations have been carried out into the light stability of nifedipine in toluene [3, 5], ethyl acetate [4], dilute hydrochloric acid [7] and plasma [5] under laboratory light. However, in this paper we report the results of our investigations into the light protective effect of whole blood as compared to plasma and water.

MATERIALS AND METHODS

Materials

Nifedipine (BAY a 1040) and internal standard nitrendipine (BAY e 5009) were donated by Bayer U.K.

Toluene was HPLC grade (BDH, Poole, U.K.). All other chemicals were analytical-reagent grade. Blood was obtained from date-expired transfusion blood.

Apparatus

A Varian Vista 6000 gas chromatograph (Varian, Walnut Creek, CA, U.S.A.) was equipped with an electron-catpure detector (^{63}Ni) and autoinjector (Varian Series 8000).

The column fitted was a fused-silica open tubular SE 30 column, 12 m × 0.33 mm I.D. (Scientific Glass Engineering, Milton Keynes, U.K.). The carrier gas, helium, flowed at 2.5 ml/min and nitrogen make-up gas at 30 ml/min.

The injection mode was splitless on a split/splitless fitting, with flushing of excess solvent after 0.7 min, obtained by opening the split.

Temperature settings used: detector 300°C, injector 250°C; temperature programme: 100°C to 200°C at 40°C/min, 200°C to 260°C at 10°C/min, hold for 1 min. The attenuation was 16 × 10.

The sodium lamp for sample preparation was from Gallenkamp (London, U.K.).

Procedure

All sample preparation was carried out in a darkened room under yellow sodium light to prevent photodecomposition. Stock solutions of 1 mg/ml nifedipine and 1 mg/ml internal standard (nitrendipine) in methanol were stored at 4°C in glass wrapped in aluminium foil.

From these solutions, aqueous 100 ng/ml nifedipine and 1 μg/ml internal standard were made up fresh for each assay. For a calibration series (0—100 ng/ml) aliquots of the nifedipine solution were added to 1 ml of blank plasma along with distilled water to bring the volume to 2 ml. To 1 ml of plasma containing nifedipine 1 ml of distilled water was added. Then 0.1 ml of internal standard solution was pipetted into all the tubes followed by 0.1 ml of ammonia (0.88 specific gravity) and 1 ml of toluene.

The tubes were shaken for 5 min prior to centrifugation at 500 *g* for 10 min. A 0.2-ml aliquot of the clear toluene layer was transferred to an autoinjector vial and 1 μl injected into the gas chromatograph.

A calibration curve of nifedipine concentration (ng/ml) in standard versus ratio of nifedipine peak height to internal standard peak height was plotted.

Nifedipine degradation

The breakdown rate of nifedipine in laboratory light was examined in distilled water, plasma and whole blood. These three media were spiked with nifedipine at levels of 25, 50 or 100 ng/ml from an aqueous 10 μg/ml nifedipine solution, and made up to 10 ml.

These solutions were exposed to laboratory light (a mixture of daylight and fluorescent light) for 4 h. At zero time and subsequently after 0.5, 1, 2 and 4 h, 1-ml aliquots were taken for nifedipine analysis. Controls of 50 ng/ml nifedipine in whole blood or plasma protected from the light were run each time.

To assess statistically significant differences between populations the Students' t test was used.

RESULTS

Typical chromatograms obtained using this assay method are shown in Fig. 1. The following nifedipine metabolites were checked for interference: nitropyridine metabolite (BAY b 4759), carboxylic acid form known as metabolite I (BAY o 2820) and lactone form known as metabolite II (BAY h 2228). The drugs investigated were: propranolol, metoprolol, quinidine, bendrofluazide, frusemide, diazepam and nitrazepam. Interference at nifedipine or internal standard retention times was not seen with any of these compounds.

The calibration curve is linear from 0 to 150 ng/ml, which amply encompasses the therapeutic range. Linear regression analysis performed on ten calibrations over a period of one month yielded the following results: $r = 0.996$ (S.D. = 0.004); slope = 0.0096 (S.D. = 0.0016) and y axis intercept = 0.0089 (S.D. = 0.0057). Stock solutions of nifedipine and internal standard in

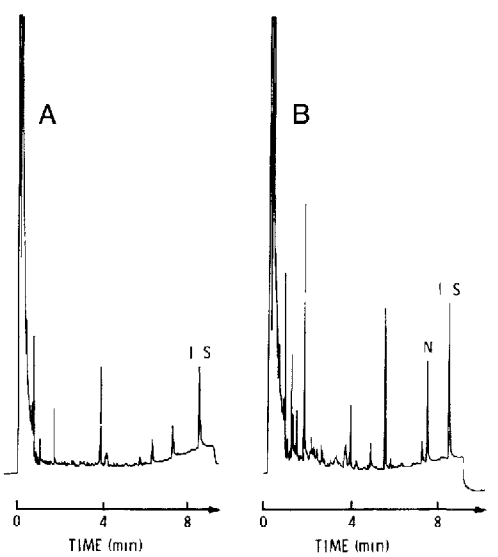

Fig. 1. Gas chromatogram of (A) blank plasma containing internal standard, nitrendipine, (B) patients' plasma containing 60 ng/ml nifedipine and internal standard. Peaks: I.S. = internal standard; N = nifedipine.

TABLE I

INTRA- AND INTER-ASSAY VARIATION OF GLC METHOD

Nifedipine added to plasma (ng/ml)	Intra-assay varation			Inter-assay varıation		
	n	Mean value (ng/ml)	C.V.* (%)	*n*	Mean value (ng/ml)	C V.* (%)
5	5	6.0	8.2	6	6.2	10.0
50	6	49.0	6.0	9	46.9	7.5
100	5	102.0	6.1	9	99.5	4.9

*C.V. = Coefficient of variatıon.

methanol used for the calibration curve were stable for at least two months.

The extractıon efficiency of nifedipine was checked at concentrations of 5, 50 and 100 ng/ml, which gave values of 105%, 70% and 69.5%, respectively.

The accuracy and precision of the method above, below and at the therapeutic range are illustrated in Table I. To assess inter-assay variation each nifedipine concentration was analysed in triplicate on several occasions. The nifedipine detection limit of this assay is approximately 0.5 ng/ml.

Photodecomposition of nifedipine

The light-protective effect of whole blood ıs very marked, allowing only an 11% decrease in nifedipine level due to photodegradation over 4 h compared to a 62% decrease in plasma and 79% decrease in aqueous solution (see Fig. 2). Controls of whole blood or plasma wrapped ın foil show no reduction in nifedipine throughout the 4 h.

For 25, 50 and 100 ng/ml nifedipine in whole blood the breakdown is non-exponential and non-linear, however, in plasma and distilled water breakdown of nifedipine is exponential. This was established using a curve fitting programme in which photodecomposition corresponded to eqn. 1.

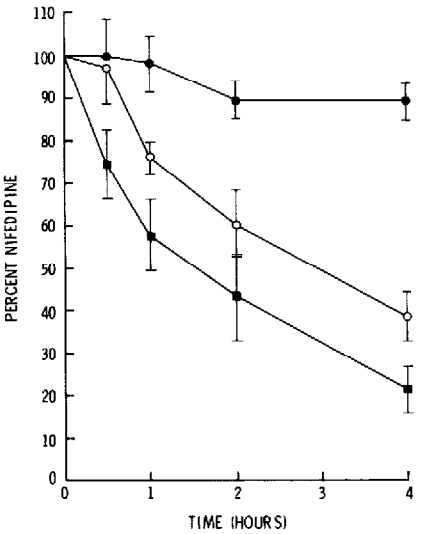

Fig. 2. Rate of photodegradation in whole blood (•), plasma (○) and distilled water (▪), showing standard error bars.

$$C = C_0 e^{-kt} \quad (1)$$

C_0 = Percent nifedipine at $t = 0$; C = percent nifedipine at time t; k = degradation rate constant.

Rate of degradation (k) was calculated from the programme and half-life ($t_{1/2}$) obtained from eqn. 3.

$$t = (2.303/k) \cdot \log(C_0/C) \quad (2)$$

which becomes

$$t_{1/2} = 0.693/k \quad (3)$$

Plasma and distilled water containing 50 ng/ml nifedipine were analysed simultaneously on five separate occasions and their photodegradation was monitored. From this, the rates of decay and half-life were calculated and the results are shown in Table II. There is a significant difference in degradation rates for each matrix ($p < 0.05$) although there is no significant difference between the half-lives.

TABLE II

EXPONENTIAL PHOTODECOMPOSITION RATES FOR NIFEDIPINE

Matrix	Degradation rate constant (h^{-1})		Half-life (h)	
	Mean ± S.E.	Range	Mean ± S.E.	Range
Plasma	0.25 ± 0 04	0.15—0.38	3.04 ± 0.5	1 8 —4.6
Aqueous	0.54 ± 0.11	0.20—0.88	1.64 ± 0.5	0.78—3.46

DISCUSSION

Capillary GLC has advantages over packed-column GLC for two reasons: firstly, the inert fused-silica capillary column produces sharp peaks which are well resolved and hence give high sensitivity. Secondly, the temperature programme beginning at 100°C allows exploitation of the solvent-trapping effect, improving peak shapes by concentrating the sample into a sharp band behind the solvent, which has condensed on entering the column.

The sample preparation method used here is simple and quick as nifedipine is injected into the GLC system without any prior derivatisation. In a previous method reported [8] the nifedipine was oxidized to its light-tolerant nitropyridine form to eliminate problems with light sensitivity. However, this method was not specific as the nitropyridine form occurs as a metabolite in human plasma [9]. During our preliminary development we tested this method but found it to have low day-to-day reproducibility on plasma samples.

Photodecomposition of nifedipine

The large variability in photodecomposition rate is due to a daily variation in the intensity of light falling on the samples, due to laboratory light being a mixture of sunlight and fluorescent lighting. From the curves of breakdown rate shown in Fig. 2 the red blood cells seem to be acting as a light filter. The

rate of drug breakdown is less than 2% per hour compared to 25% per hour in plasma. However, plasma has a small light-protective effect as the rate of decomposition in plasma is significantly lower than that in water ($p < 0.05$). This is possibly due to protein binding of nifedipine and also the opaque nature of plasma.

These curves show that once blood containing nifedipine has been centrifuged it is necessary to avoid light contact as plasma can lose as much as 7% of its nifedipine over 15 min, whereas whole blood could be safely left in the light for this timespan. This conclusion has clinical applications in two areas. Firstly, when blood is taken from patients for nifedipine analysis, it is not necessary to cover the syringe in aluminium foil or wrap the samples in foil as no drug photodecomposition will occur immediately. Care need only be taken once the sample has been centrifuged, then the plasma must be protected from the light. Secondly, in haemodialysis when the patients' blood is exposed to light it is likely there would be little nifedipine degradation or production of its photodecomposition products.

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