Pergamon

0731-7085(94)00100-6

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 12, pp. 1529–1535, 1994
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0731-7085/94 $7.00 + 0.00

Photostability determination of commercially available nifedipine oral dosage formulations

JOHN S. GRUNDY, RAHEEM KHERANI and ROBERT T. FOSTER*

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2N8, Canada

Abstract: Nifedipine (NIF), a 1,4-dihydropyridine calcium channel antagonist, undergoes photodegradation to dehydronifedipine (DNIF) upon exposure to ultraviolet (UV) light and to the nitroso analogue of dehydronifedipine (NDNIF) when exposed to sunlight. NIF photodegradation products do not contribute to clinical activity, thus the content of NIF must remain uniform between equipotent formulations. Large differences in light stability between bioequivalent NIF products could potentially result in the therapeutic failure of unstable preparations. Consequently, if large photostability differences do exist between NIF preparations, product substitution may not be warranted. The light stability of 10 intact immediate- or controlled-release oral NIF formulations, obtained from several European and North American manufacturers, was studied using direct continuous artificial sunlight exposure extending over a 12-week period. The content of both NIF and NDNIF for each product was measured to determine the extent of photodecomposition using a specific and sensitive reversed-phase high pressure liquid chromatographic (HPLC) method. In addition, NIF photodegradation was measured using both pure NIF powder and methanolic NIF solution to determine the effectiveness of the artificial sunlight source used in this study. After 12 weeks of artificial sunlight exposure, less than 3% of NDNIF (w/w initial NIF content) was present in each of the 10 tested dosage forms. Photodegradation was greater than 10% (w/w initial NIF content) in ~5–10 min (mean $t_{1/2}$ = 31 min), and in ~24 h (mean $t_{1/2}$ = 7.7 days) of artificial sunlight exposure for methanolic NIF solution and pure NIF powder samples, respectively. Therefore, the tested NIF formulations all appear to be photostable up to at least 12 weeks of continuous artificial sunlight exposure, compared with pure NIF powder and methanolic NIF solution. It is concluded that if therapeutic failures or pharmacodynamic differences between the tested NIF formulations were observed, photoinstability as a major contributory factor would be unlikely.

Keywords: *Artificial sunlight; GITS; HPLC; nifedipine; photodegradation; photostability.*

Introduction

Nifedipine (NIF), 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester (Fig. 1A), is the prototype compound of the dihydropyridine class of calcium channel antagonists. NIF is a selective arterial dilator, and is frequently used for the treatment of hypertension, angina pectoris and other cardiovascular disorders [1]. In human, NIF is rapidly metabolized by oxidative mechanisms to dehydronifedipine (DNIF, Fig. 1B), which is further metabolized to more polar compounds [2–6].

NIF is highly sensitive to photo-oxidation, changing in colour from yellow to brown upon exposure to light [7]. Exposure of NIF to ultraviolet (UV) light results in the formation of DNIF. Upon exposure to ultraviolet–visible (UV/VIS) light (i.e. daylight), NIF degrades to the nitroso-analogue of dehydronifedipine (NDNIF, Fig. 1C) [8]. NIF photodegradation products have little or no pharmacological activity [9, 10]. Matsuda *et al.* [11] reported that NIF undergoes degradation to four products after exposure to fluorescent and mercury vapor light sources, three of these (including DNIF) were produced in minor quantities and the fourth compound, NDNIF, was identified as the sole major photodecomposition product. Therefore, quantitative photodegradation analysis of NIF formulations only requires the accurate detection and quantification of NIF and its major photodecomposition product NDNIF as presented in this report.

Several studies in the past have been conducted to determine the photostability of NIF in solution and in the solid state, including photostability of pulverized NIF tablet powders [3, 5, 11–13]. This report is one of the few studies to compare NIF stability in intact commercial oral NIF formulations and, to our knowledge, is the first to report the photo-

* Author to whom correspondence should be addressed.

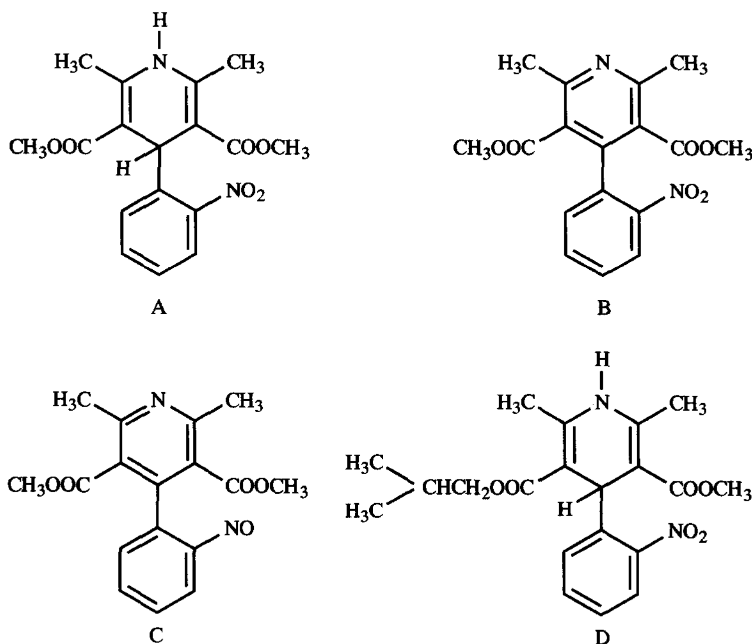

Figure 1
Structures of (A) nifedipine (NIF), (B) dehydronifedipine (DNIF), (C) nitroso-analogue of dehydronifedipine (NDNIF) and (D) nisoldipine (I.S.).

stability of prolonged-action (PA) and gastro-intestinal therapeutic system (GITS) NIF products [14, 15].

Official USP guidelines for NIF capsules state that the measured amount of NIF in a commercial product be within 90–110% of the labelled quantity [16]. Manufacturers of NIF products utilize light resistant coatings and/or packaging to minimize photodegradation of oral NIF preparations from inadvertent light exposure. Few quantitative studies on the light transmissive properties of different light protective tablet film coatings or protective packaging have been done [17]. Thus, differences in degree of light protection may exist between different brands and/or formulation types of NIF products. Long term exposure, several weeks or longer, to direct sunlight may occur if NIF formulations are improperly stored by patients. Inadequate storage conditions may potentially contribute to a decrease in clinical efficacy of light sensitive NIF products. Consequently, if differences in photostability between NIF products are present, product substitution may not be warranted. It is the purpose of this study to quantify NIF and NDNIF content in different NIF formulations subjected to long-term artificial sunlight exposure, and to determine if they exhibit similar and/or satisfactory resistance to photodegradation.

In this paper, we report the photodegradation of NIF, after exposure to an artificial sunlight source, in 10 different NIF formulations utilizing a specific and sensitive HPLC assay to detect NIF and NDNIF content. The present study also compares authentic NIF powder and methanolic NIF solution photodegradation to the 10 tested NIF formulations.

Experimental

Chemicals

NIF was purchased from the Sigma Chemical Co. (St Louis, MO, USA). Internal standard (nisoldipine, I.S., Fig. 1D), NDNIF, DNIF (Bay b 4759), and the carboxylic acid metabolite (Bay *o* 2820) were kindly provided by Miles Canada Inc. (Etobicoke, Ontario, Canada). Methanol, water and triethylamine (TEA) were obtained from Mallinckrodt (Paris, KY, USA). Iso-octane, methyl-t-butyl ether (MTBE), sodium hydroxide and glacial acetic acid were purchased from BDH (Toronto, Ontario, Canada). Chloroform was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All reagents and chemicals were either analytical or HPLC grade.

Standard solutions

Stock solutions, 100 $\mu g\ ml^{-1}$ (as base) of

both NIF and NDNIF and 50 μg ml^{-1} (as base) of I.S., were prepared in methanol. Solutions were kept protected from light with aluminum foil, stored at 4°C and were found to maintain their original concentrations of NIF or NDNIF, respectively, for a period of at least 3 months. Solutions containing 10 μg ml^{-1} of NIF and NDNIF in methanol (solutions 1 and 2, respectively) were prepared daily from the respective stock solutions.

Formulations

Ten nifedipine formulations obtained from several manufacturers in North America and Germany were studied. Formulations tested included: six prolonged-action (PA) 20-mg tablets [Adalat PA, Miles (Canada); Nifelat 20 retard, TAD (Germany); Nife-Wolff 20 retard, Vinces (Germany); Nifical tablinen retard, Sanorania (Germany); Nifehexal retard, Hexal Pharma (Germany); and Pidilat retard, Giulini Pharma (Germany)], one 20-mg PA capsule [Nifedipin AL 20, Aliud Pharma (Germany)], two immediate-release (IR) 10-mg capsules [Apo-Nifed, Apotex (Canada); Novo-Nifedin, Novopharm (Canada)] and one gastrointestinal therapeutic system (GITS) 60-mg tablet [Adalat XL, Miles (Canada)].

Irradiation test

The irradiation test employed utilized a full-spectrum lamp (20 Watt, 900 lumens, vacuum tube length 59 cm, Commercial Lighting Products Ltd, Edmonton, Alberta, Canada) placed 22 cm from nifedipine samples. Samples were placed on aluminum foil to allow for more uniform irradiation. Irradiation was conducted inside a cabinet to protect samples from extraneous light. Each formulation was irradiated (24 h day^{-1}) by the artificial sunlight source for a period ranging from 0 to 12 weeks. Samples were taken at 0, 1, 2, 4, 6, 8, 10 and 12 weeks ($n = 6$ replicate samples/time interval). In addition, a total of 11 × 1 ml methanolic NIF solution samples (100 μg ml^{-1}) placed in 5-ml clear glass vials were irradiated for a period of 0–360 min (aliquots for analysis were taken at 0, 5, 10, 15, 20, 30, 45, 60, 120, 240 and 360 min, $n = 3$ replicates). A total of 7 × 10 mg nifedipine powder samples placed in 5-ml clear glass vials were also irradiated for a period of 0–12 days (aliquots for analysis were taken at 0, 1, 2, 3, 7, 10 and 12 days, $n = 3$ replicates). Sample irradiations were conducted at ambient temperature.

Sample preparation

Prolonged-action (PA) tablet and capsule-pellet formulations. Each PA tablet or the contents of a PA capsule were crushed into fine particles in a disposable 20-ml paper cup using a small glass pestle. The crushed particles were placed in a small microcentrifuge tube and 1 ml of chloroform was added. The resulting mixture was vortexed for 15 s and centrifuged for 5 min. Vortexing of NIF samples was accomplished with a Genie 2 mixer (Fisher Scientific, Edmonton, Canada) and centrifugation was performed with a microcentrifuge (Microfuge E, Beckman, Palo Alto, CA, USA). From the supernatant, 25 μl of solution was placed in a small glass vial, diluted to a final volume of 1.5 ml with chloroform and vortexed for 15 s. A 30-μl volume of the diluted solution was added to a clean dry 13 × 100 mm glass test tube containing 10 μg (200 μl) of I.S. The sample was then evaporated to dryness (low heat) in a concentrator–evaporator (Model SC 100 Savant Speed Vac concentrator–evaporator, Emerston Instruments, Scarborough, Canada). To the resulting residue was added 200 μl of mobile phase and the solution was vortex-mixed for 15 s. Aliquots of 10 μl were injected onto the HPLC column.

Liquid filled immediate release capsules. A 100-μl volume of solution containing NIF was withdrawn from each NIF capsule using a 21 gauge needle and a 1-ml syringe and diluted 100 times with chloroform. A 25-μl aliquot of this was placed in a small glass vial, diluted to a final volume of 1.5 ml with chloroform and vortexed for 15 s. From this dilute solution, 30 μl was added to a clean dry 13 × 100 mm glass test tube containing 10 μg (200 μl) of I.S. Further sample evaporation and preparation/injection was as described per PA tablets and PA capsules.

Gastrointestinal therapeutic system (GITS) tablets. Single 60-mg GITS tablets were added to 50 ml of chloroform in a stainless steel blender (Model 702CR, Rotor Electric Co. Ltd) and homogenized for 10 min. A 100-μl aliquot of the resulting mixture was placed in a small glass vial and diluted to a final volume of 1 ml with chloroform. A total of 83 μl (containing 10 μg of NIF) of the diluted mixture was placed in a clean dry 13 × 100 mm glass test tube containing 10 μg (200 μl) of I.S.

Sample evaporation and further preparation/injection was as described above.

Methanolic NIF solutions. Three 10-μg (100-μl) samples were withdrawn from each vial at the sampling times previously described. Each of these samples was placed in a clean dry 13 × 100 mm glass test tube containing 10 μg (200 μl) of I.S. Sample evaporation and further preparation/injection was as described above.

NIF powder samples. Each irradiated NIF powder sample was diluted with 1 ml of methanol (10 mg ml^{-1}), then further diluted with methanol to 100 μg ml^{-1}. Three 10-μg (100-μl) samples from each time point were placed in a clean dry 13 × 100 mm glass test tube containing 10 μg (200 μl) of I.S. Sample evaporation and further preparation/injection was as described previously.

Chromatography and instrumentation

Determination of NIF and NDNIF utilized a previously reported HPLC method [18]. Briefly, the HPLC system employed consisted of a Model 600E solvent delivery system, a Model 717 autosampler and a 486 tunable UV/VIS absorbance detector set at 350 nm. Analytical separation was accomplished using a Nova-Pak 8 × 100 mm radial pack column containing 4-μm C8 packing material (Waters, Mississauga, Ontario, Canada). Mobile phase flow rate was 1.1 ml min^{-1} and consisted of methanol–water (65:35 v/v) adjusted to approximately pH* 4.0 with acetic acid and TEA as 1 and 0.03% final concentration, respectively. The mobile phase was continuously degassed with helium (100 ml min^{-1}). Sample preparation and analysis were conducted at room temperature under sodium lamps. Sample spectra were recorded using either a Waters 745B integrator or Millennium 2010 chromatography manager software installed on an NEC 486/33i computer (Waters, Mississauga, Ontario, Canada).

Quantification

Calibration curves were constructed by plotting the peak area ratios (NIF/I.S.) or (NDNIF/I.S.) vs their corresponding added concentrations. Calibration curve concentration ranges used in testing of irradiated NIF formulations were from 0 to 35 μg and from 0 to 750 ng for NIF and NDNIF, respectively. Calibration curve concentration ranges used in testing of methanolic NIF solution and NIF powder were from 0 to 35 μg, and from 0 to 15 μg for NIF and NDNIF, respectively. An unweighted least squares linear regression analysis was performed to generate a best-fit regression line for each compound. Calculation of NIF powder and solution $t_{1/2}$ were determined from the slope of the log %NIF vs time plots (i.e. $t_{1/2} = 0.693/K$). Data are presented as mean ± SD.

Mass spectrometry

Authentic NIF and NDNIF reference standards, and NDNIF prepared from exposure of 2 mg ml^{-1} methanolic NIF solution to artificial sunlight for 4.5 h, were chromatographed and the eluent corresponding to the assumed NIF and NDNIF peaks were collected for analysis. Samples were evaporated using a Speed Vac evaporator–concentrator and the residues subjected to high-resolution electron impact mass spectral analysis (AEI, MS9, Manchester, UK) via direct insertion probe at 70 eV ionizing potential.

Other compounds tested

Two additional NIF metabolites/photodegradation products were tested for interference using the chromatographic method employed for this study: (1) DNIF (Bay b 4759) and (2) the carboxylic acid metabolite (CAM, Bay *o* 2820).

Results and Discussion

In the present study, a measure of the percentage NDNIF content (w/w initial NIF content), in intact commercial NIF formulations, was determined after continuous exposure to artificial sunlight. A maximum of 12 weeks continuous artificial sunlight irradiation was evaluated. Natural sunlight exposure of commercial NIF preparations could be of the magnitude studied when stored incorrectly. The use of artificial sunlight in this study overcomes the potential problems inherent when using natural sunlight such as daytime, regional, weather and seasonal variability.

Figure 2 depicts typical chromatograms (each spiked with 10 μg I.S.) of: a blank sample (a); a sample spiked with authentic NIF and NDNIF (b); a sample of NIF methanolic solution irradiated for 45 min (c); and a commercial 20-mg NIF PA capsule irradiated

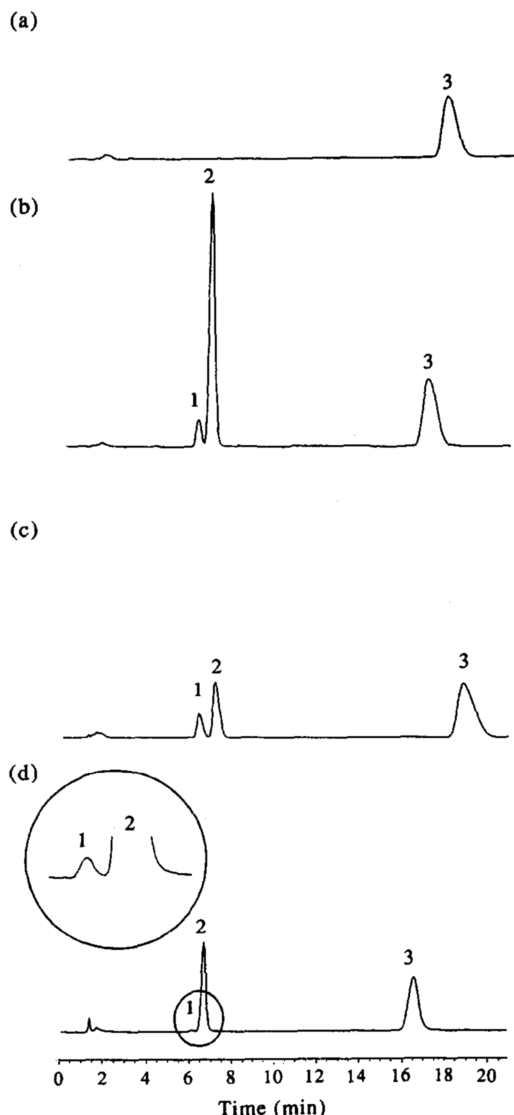

Figure 2
Chromatograms of: (a) a blank containing 10 μg I.S.; (b) authentic NIF, NDNIF and I.S. (15, 5 and 10 μg, respectively); (c) methanolic NIF solution (100 μg ml^{-1}) irradiated by the artificial sunlight lamp for 45 min; NDNIF, NIF, and I.S. peaks correspond to 5.7, 4.3, and 10 μg, respectively; and (d) a commercial 20 mg NIF PA capsule irradiated for 12 weeks (includes an enlarged view of the small NDNIF peak). Peak identification: (1) NDNIF; (2) NIF; and (3) I.S.

for 12 weeks (d). UV/VIS absorption of all compounds was measured at a wavelength of 350 nm as greater accuracy at this wavelength was previously reported, for the simultaneous determination of both NIF and NDNIF concentration, compared to lower UV wavelengths [4, 13]. In our results, a peak corresponding to NIF eluted at approximately 7.0 min, NDNIF eluted at approximately 6.0 min and I.S. at approximately 17 min. Adequate resolution ($R > 1.5$) of the three peaks of interest was observed using the chromatographic system employed. Calibration curves were linear over 1–35 μg, 50–750 ng and 0.5–15 μg for NIF (all tests), NDNIF (formulation tests) and NDNIF (solution/powder tests), respectively. Observed r^2 values were >0.99 for all calibration curves. Additionally, two known human NIF metabolites, DNIF (also a NIF photodegradation product after UV light exposure) and the carboxylic acid metabolite (CAM) were tested for interference with the compounds of interest using identical HPLC conditions. No interference was observed between any of the compounds tested (peaks obtained at ~2 and ~5 min for CAM and DNIF, respectively; chromatograms not shown). DNIF and CAM peaks were not detected in any formulation or solution/powder analyses conducted in this study.

Mass spectra of both NIF and NDNIF residues collected from evaporated peak eluents assisted in the assignment of peak identity. Mass spectra of authentic NIF produced major fragments at m/z 346 (10%), 329 (100%) and 284 (62%). Mass spectra of authentic NDNIF produced major fragments at 328 (47%), 298 (11%) and 269 (100%). NIF obtained from the assumed NIF peak eluent from an extracted NIF tablet, and NDNIF obtained from the assumed NDNIF peak eluent of a methanolic NIF solution previously exposed to artificial sunlight (>4 h), produced mass spectra consistent with those found for authentic NIF and NDNIF, respectively.

Figure 3 shows photodegradation plots of NIF powder (A) and NIF methanolic solution (B) after artificial sunlight irradiation. Photodegradation of NIF powder, measured as percentage loss of NIF, exceeded 10% in 24 h ($t_{1/2}$ = 7.7 days). Photodegradation of NIF methanolic solution exceeded 10% in approximately 5–10 min and was essentially complete within 4 h ($t_{1/2}$ = 31 min). These results are consistent with those of previously published NIF powder and alcoholic solution degradation studies [8, 11, 13]. Berson and Brown [7] reported that NIF photo decomposition appears to follow zero order kinetics until the reaction is ~60% complete, after which the rate decreases in a first order fashion. This was attributed to inhibition of the NIF degradation reaction by NDNIF. Other studies have used first order kinetics or more complex models to describe NIF degradation [17, 19]. No attempt

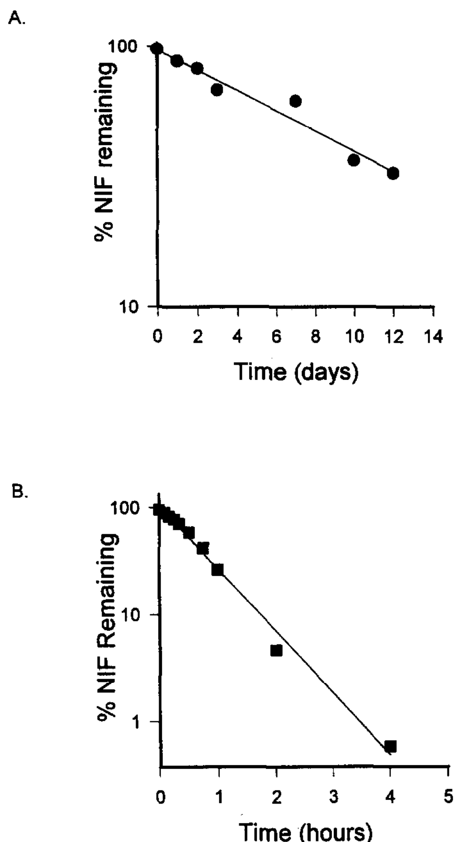

Figure 3
NIF photodegradation curves. (A) NIF powder, (B) methanolic NIF solution (100 μg ml^{-1}).

was made in this analysis to further define NIF degradation kinetics, as the primary objective was to compare intact commercially available NIF formulation stability. Nevertheless, apparent first order degradation was observed for NIF degradation kinetics.

Exposure of methanolic NIF solution to either natural sunlight (using midday sun) or the artificial sunlight source used in this study, yielded 100% decomposition of NIF after 4 h in both situations. It seems reasonable, therefore, to conclude that the artificial sunlight lamp adequately approximated natural sunlight conditions for the purposes of this study.

The percentage of NDNIF (w/w initial intact NIF) present in all 10 tested NIF formulations, irradiated for up to 12 weeks by artificial sunlight, was found to be very low (~1–3%). Table 1 summarizes photodegradation data for each formulation after 0, 2 and 12 weeks of artificial sunlight exposure. The average percentage of NDNIF found in the formulations was 1.1, 1.29 and 1.54% for 0, 2 and 12 weeks, respectively. The intra-formulation standard deviation of percentage NDNIF ($n = 6$ samples per time period) was <0.5% in all cases. The maximum recorded percentage of NDNIF was 2.64 ± 0.45%, found in the NIF 20 mg PA capsule after 12 weeks continuous irradiation. Inter-formulation photodegradation differences were not determined since these variations, although possibly statistically significant, are likely clinically insignificant. Rates of NIF degradation for individual formulations were not calculated because of the relatively small degree of NIF decomposition observed in this study.

Results obtained in this analysis indicated that all the tested NIF formulations did not undergo appreciable NIF decomposition (>10%) even after 12 weeks continuous artificial sunlight exposure. All samples tested were within the USP requirements. No

Table 1
Nifedipine formulation photodegradation data

Formulation	Dosage form	Strength	Percentage NDNIF (w/w initial NIF content)		
			0 Weeks	2 Weeks	12 Weeks
Adalat XL	GITS tablet	60 mg	1.45 ± 0.28	N/A*	1.18 ± 0.09
Adalat PA	PA tablet	20 mg	1.0†	1.09 ± 0.08	1.28 ± 0.17
Nifelat 20 retard	PA tablet	20 mg	1.0†	1.07 ± 0.04	1.77 ± 0.14
Nife-Wolffe 20 retard	PA tablet	20 mg	1.0†	1.18 ± 0.13	0.81 ± 0.10
Pidilat retard	PA tablet	20 mg	1.0†	1.0†	1.0†
Nifehexal retard	PA tablet	20 mg	1.0†	1.40 ± 0.09	2.03 ± 0.33
Nifical retard	PA tablet	20 mg	1.0†	1.0†	1.0†
Nifedipin AL 20	PA capsule	20 mg	1.45 ± 0.12	1.81 ± 0.20	2.64 ± 0.45
Apo-Nifed	IR capsule	10 mg	1.0†	1.20 ± 0.08	1.21 ± 0.09
Novo-Nifedin	IR capsule	10 mg	1.0†	1.86 ± 0.12	2.51 ± 0.24

* Data not available.
† Insufficient photodegradation product content to accurately report % NDNIF, however, values were estimated as approximately 1.0 ± 0.5%.

clinically significant differences in degree of light protection were detected in any of the NIF formulations tested in this study.

In conclusion, based on the present study results, if therapeutic failure or pharmacodynamic differences of the tested NIF formulations are observed, it is unlikely that a photostability difference would be a primary contributory factor.

Acknpwledgements — This study was supported by Miles Canada Inc. (Etobicoke, Ontario, Canada). J.S. Grundy was supported, in part, by a studentship from Merck Frosst Canada Inc. (Dorval, Quebec, Canada).

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