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Original Article

Photostability Determination of Commercially Available Nifedipine Oral Dosage Forms in Iran

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Abstract

Nifedipine (NIF) a 1, 4-dihydropyridine calcium channel antagonist, undergoes photodegradation to nitroso analogues of dehydronifedipine (NDNIF) when exposed to sunlight. Photodegradation products of NIF have no clinical activity, so different formulations of NIF must remain unchanged. If NIF preparations become unstable in exposure to light, they could cause therapeutic failure. The present study was carried out in order to investigate the photostability of commercially available NIF products, in Iran. Three oral NIF formulations available in Iran were studied using indirect sunlight (daylight) and continuous artificial light exposure extending over a period of 12 weeks. The extent of photodecomposition of NIF was determined using a specific reversed phase high performance liquid chromatography (HPLC) method. NIF photodegradation was measured using both pure NIF powder as well as a methanolic NIF solution to determine differences in the effectiveness of artificial light and natural indirect sunlight sources used in this study. All the tested NIF formulations were likely to be photostable up to at least 12 weeks of continuous artificial and natural day light exposure, compared with pure NIF powder and methanolic solution. Photodegradation of NIF powder and methanolic solution exposed to indirect sunlight was faster than the artificial light.

Keywords: Nifedipine; Photostability; HPLC; Daylight; Artificial light.

Introduction

Nifedipine (NIF), 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester (Figure 1), is the prototype compound of the dihydropyridine class of calcium channel antagonists. Calcium antagonists inhibit the influx of calcium ion through plasma membrane channels and thus dilate vascular smooth muscle contraction. NIF is a selective arterial dilator, and is used for the treatment of hypertension, angina pectoris, and other cardiovascular disorders (1-4). The exposure of some drugs to light leads to photodecomposition. These drugs undergo important chemical changes, accompained by alternation in their activities and in some cases total loss of their therapeutic activity (5). NIF is very highly sensitive to photooxidation. NIF exposure to ultraviolet-visible irradiation produces both aromatization in the dihydropyridine moiety (turning it into a pyridine ring) and a reduction of nitro group in to nitroso groups (NDNIF). In addition, its exposure to ultraviolet light produces dehydronifedipine (DNIF) (6-9) (Figure 1).

NIF photodegradation products have no pharmacological activity. Several studies related to its photodecomposition have been reported (10-13) but to our knowledge this research is the first one to report the

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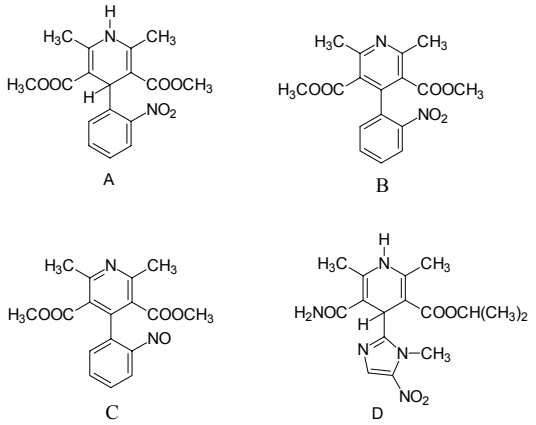

Figure 1. Structures of **A:** Nifedipine(NIF), **B:** Dehydronifedipine(DNIF), **C:** Nitroso-analogue of dehydronifedipine (NDNIF), **D:** Internal standard

photostability of oral dosage forms used in our country, Iran.

Manufacturers of NIF products use light resistant coating and/or packing to minimize their photodegradation. Long term exposure to sunlight or artificial light may also occur if NIF formulations are improperly stored by patients. Poor storage conditions may potentially decrease clinical efficacy of NIF products (14). Differences in the degree of light protection may exist between different formulation types. In this paper, we report the photostability of commercially available NIF products, in Iran. The present study also compares photodegradation of authentic NIF powder and methanolic NIF solution obtained from three tested NIF formulations.

Experimental

NIF was purchased from Sigma-Aldrich Chemie GmbH (Deisenhofen, Germany). Methanol and chloroform were obtained from Merck (Darmstadt, Germany). All reagents were analytical or HPLC grade.

Ten mg liquid filled immediate release capsules of NIF were obtained from Zahravi Co. (Tabriz, Iran) and Apotex Inc. (Toronto, Canada); and 10 mg tablets were obtained from Toliddarou Co. (Tehran, Iran). These three formulations are the commercially available NIF products in Iran. The NIF stock solution was prepared in methanol (10 mg/ml) and standard solutions were obtained by serial dilution. The Internal standard solution (IS) was prepared (40 µg/ml) in methanol. 1 ml of IS solution was mixed with 1 ml of the tested concentrations of NIF (12.5–150 µg/ml) and 20 µl of the mixed solution was injected to HPLC.

Melting point was determined on a kofler hot stage apparatus. ^{1}H-NMR spectra was run on a Varian Unity Plus 400 MHz spectrometer. TMS was used as the internal standard. Mass spectra was measured with a Finnigan TSQ-70 spectrometer at 70 eV.

Synthesis of Isopropyl-3-amido-2, 6-dimethyl-4-(1-methyl 5-nitro 2-imidazolyl)-1,4-dihydropyrinine-5-carboxylate (Internal standard**)**

A solution of acetoacetamide (0.5g, 5mmol) in MeOH (3 ml) was added to a solution of 1-methyl-5-nitro-imidazole-2-carboxaldehyde (0.77 g, 5 mmol) and isopropyl 3-amino crotonate (0.72 g , 5 mmol) in EtOH (4 ml) and stirred constantly for a period of 5 min. The reaction was heated at reflux temperature for 14 h, cooled to 25°C. The resulting precipitate was filtered and purified by recrystalization from MeOH (yield= 48%; mp = 217-219°C) (15).

^{1}H-NMR ($CDCl_3$): δ 8.86 (brs, 1H, NH), 7.94 (s, 1H, imidazole H-4), 5.14(s, 1H, C_4-H), 4.21(s, 3H, N-CH_3), 4.12(m, 3H, CO_2CH & NH_2), 2.25(s, 6H, C_2-CH_3 & C_6-CH_3), 1.28 &1.18 (twod, J=7.2 Hz, 3H each, CH (CH_3)$_2$).

MS: m/z (%) 364(M^+ +1, 1.8), 305(4), 291(7), 259(68), 238(100), 210(29)

Irradiation test

For artificial light irradiation, a 40 W tungsten lamp was used. NIF samples were placed 50 cm from the lamp in a cabinet (1 m × 1 m × 0.75 m). Protected samples from extraneous light were placed in aluminum foils. Exposure to indirect sunlight was also used during the spring months in Shiraz to compare the efficacy of artificial light and natural room daylight in photodecomposition of NIF. Samples were irradiated from 0-12 weeks in artificial light and indirect sunlight. Samples were collected at 0, 1, 2, 4, 6, 8, 10 and 12 weeks intervals (n=3). NIF powder samples (10 mg) were placed in 10 ml clean glass vials, irradiated from 0-12 days and samples were collected at 0, 1, 2, 3, 7, 10 and 12 days. Also a total of 11 × 1 ml methanolic NIF solution samples (10 µg/ml) were placed in 5 ml clear glass vials and irradiated for a period of 0-360

min. Samples were taken at 0, 5, 10, 15, 20, 30, 45, 60, 120, 240, 360 mins. The experiments were conducted at ambient temperature (14).

Sample preparation

Nifedipine tablets: Three NIF tablets were crushed into fine particles and a quantity equivalent to 10 mg of NIF was placed in a centrifuge tube and 2 ml of chloroform was added. The mixture was vortexed for 30 s and centrifuged for 5 min. Twenty µl of supernatant was drawn by Hamiltonian syringe and added to a tube containing 100 µl of IS solution (40 µg/ml). This solution was evaporated to a dry residue under Nitrogen stream. Two hundred µl of mobile phase was added to the residue and vortexed for 10 s. Aliquots of 10 µl were injected to HPLC (14).

Nifedipine softgel capsules: a 20 µl volume solution from each NIF capsule was withdrawn using a 25 µl Hamiltonian syringe and was diluted with 0.5 ml of chloroform. One hundred µl of IS solution (40 µg/ml) was added and mixed on a vortex for 30 s. Further sample preparation and evaporation was as described for tablets.

Nifedipine powder samples: each of the irradiated NIF powder samples were diluted with 2 ml of chloroform and vortexed for 20 s. Twenty µl of this solution was mixed with 100 µl of IS solution (40 µg/ml), and dried over nitrogen stream, then 1 ml of mobile phase was added and vortexed for 10 s, Finally, 10 µl of this solution was injected to HPLC.

Nifedipine methanolic solution: One hundred µl of IS was added to the vials containing 1 ml of NIF (100 µg/ml) and vortexed, and then 10 µl of the solution was injected to HPLC.

Chromatography and instrumentation

Analytical separation was accomplished using a Bondapack C_{18} (250×4.6 mm) column. Mobile phase flow rate was 1.5 ml/min and consisted of methanol/water (60/40). It was continuously degassed with Helium (60 ml/min). The HPLC system employed consisted of a model 604 solvent delivery system and a 486 tunable uv/vis absorbance detector set at 350 nm (Waters). Sample preparation and analysis were conducted under sodium lamp and at room temperature.

Statistical analysis

Data were analyzed by Excel software. Paired t-test was implemented for group comparisons.

Identification of the NDNIF

0.2 mg/ml of NIF methanolic solution was exposed to indirect sunlight. At 5 min intervals 10 µl of the solution were injected to HPLC until no NIF peak was observed in chromatogram (15 min). The solution was then evaporated over nitrogen stream. The mass spectrum of the residue was obtained on a Finnigan Mat at 70 eV ionization potential.

Results and Discussion

In the present study photostability of NIF formulations available in Iran was determined after exposure to indirect natural light and continuous artificial light. NDNIF, NIF and IS were eluted at approximately 5.5, 7.0 and 11.5 min respectively. Resolution between NIF and NDNIF was adequate (R>1.5) and no buffer and pH adjustment was required with this chromatographic system. Mass spectra of photodecomposition product produced major fragments at 328 (M^+, 28%), 298 (15%), 269 (100%) and 253 (50%), that confirmed the structure of NDNIF. Figure 2 shows a typical chromatogram of a) a sample of NIF methanolic solution irradiated with artificial light for 120 min, b) a sample of NIF methanolic solution irradiated with indirect natural sunlight for 1 min and c) a commercial 10 mg NIF tablet irradiated for 12 weeks with indirect natural sunlight.

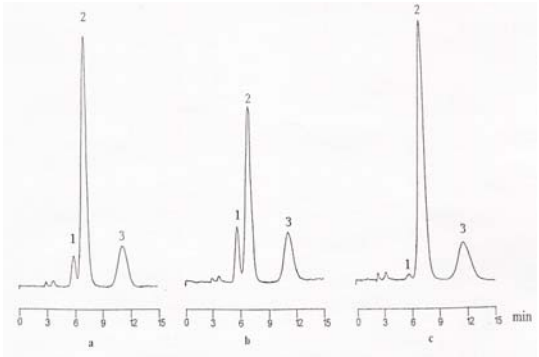

Figure 2. Chromatograms of: (a) methanolic NIF solution irradiated by artificial light for 120 min, and (b) methanolic NIF solution irradiated by natural sunlight for 1 min, and (c) a commercial 10 mg NIF tablet irradiated by natural sunlight for 12 weeks. Peak identification: (1) NDNIF; (2) NIF; (3) I.S.

Table 1. Risults obtained from the photodegradation of various nifedipine formulations by exposure to artificial light (n=3).

Formulation	Dosage form	Percentage Nif (w/w initial Nif content)		
		0 week	2 weeks	12 weeks
Toliddaru	Tablet(10mg)	99± 8	104± 2	103± 1
Zahravi	Capsule(10g)	99± 1	95± 2	99± 2
Apotex	Capsule(10g)	99± 1	101± 1	92± 8

Previous studies performed by Foster in Canada has shown that natural and 20 W tungsten artificial light have similar effects in the photodecomposition of NIF. But in our study NIF methanolic solution exposed to natural indirect sunlight was completely photodecomposed in 10 min. On the other hand photodecomposition of NIF methanolic solution exposed to artificial light exceeded 7.3 % in approximately 10 min. This result shows that the efficacy of natural indirect sunlight in photodecomposition of NIF is more than artificial light in Iran and it can be due to the fact that the intensity of natural light in Iran is more than Canada. Therefore, these results are evidence for that artificial light can not be used alone in photodecomposition studies. Photodegradaition plots of NIF methanolic solution (A) and NIF powder (B) after artificial light irradiation are shown in Figure 3. Photodegradation of NIF powder measured as the loss percentage of NIF, exceeded 5.6 % and 16.8%, in approximately 24 h after artificial and natural indirect light irradiation respectively. Our major goal in this study was to find that

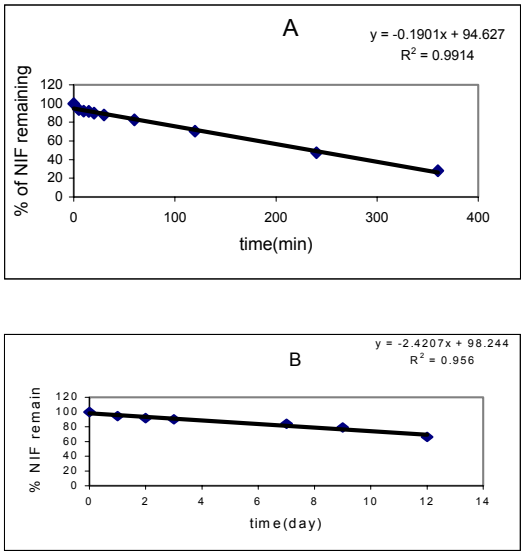

Figure 3. NIF photodegradation curves of (A) NIF methanolic solution and (B) NIF powder, irradiated by artificial light (n=3).

Table 2. Results obtained from the photodegradation of Various nifedipine formulations by exposure to natural light (n =3).

Formulation	Dosage form	Percentage of Nif (w/w initial Nif content)		
		0 week	2 weeks	12weeks
Toliddaru	Tablet(10m)	99± 8	102± 5	105±1
Zahravi	Capsule(mg)	99± 1	94± 3	95± 6
Apotex	Capsule(mg)	99± 1	97± 1	96± 7

whether manufacturers in Iran can produce NIF formulations in a photoprotective condition. For this reason three formulations, two produced in Iran, and another a commercially available form of NIF used in Iran (Apotex Inc.), as a control sample, were chosen. The percentage of NIF content (w/w initial NIF content) was measured in all three tested formulations irradiated for up to 12 weeks by artificial and natural light. Table 1 shows photodegradation data for each formulation after 0, 2 and 12 week exposure to artificial light and Table 2 shows this photodegradation data for each formulation after 0, 2 and 12 week exposure to natural indirect sunlight. Results obtained in this study showed that differences between data obtained were not significant and none of the tested NIF formulations underwent any appreciable decomposition (> 10%), even after 12 weeks irradiation.

In conclusion, based on our results it is unlikely that a photostability degradation would be the primary contributing factor, should therapeutic failure of the tested NIF formulations be observed.

Acknowledgment

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