ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 128–133

IL FARMACO

UV derivative spectrophotometric study of the photochemical degradation of nisoldipine

Valentina Marinkovic [a], Danica Agbaba [b,*], Katarina Karljikovic-Rajic [b], Jozef Comor [c], Dobrila Zivanov-Stakic [b]

[a] *Zdravlje Pharmaceutical and Chemical Industry, Quality Control Sector, 16000 Leskovac, Yugoslavia*
[b] *Faculty of Pharmacy, Department of Pharmaceutical and Analytical Chemistry, University of Belgrade, PO Box 146, 11221 Belgrade, Yugoslavia*
[c] *Vinca Institute of Nuclear Sciences, PO Box 522, 11001 Belgrade, Yugoslavia*

Received 29 July 1999; accepted 30 December 1999

Abstract

The photodecomposition of nisoldipine ((±)3-isobutyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate), whereby its 4-(2-nitrosophenyl) pyridine analogue is obtained as the photolytic product, was investigated under daylight exposure by means of UV derivative spectrophotometry. The optimal instrumental parameters (120 nm/min scan speed; 2 nm slit width; $\Delta\lambda = 10$ nm and 5 s response time) for analogue derivative spectra were established for amplitudes $^1D_{285}$ and $^2D_{291}$ (measured to the baseline) of the nitroso analogue assay, as well as for $^1D_{386}$ of the parent compound–nisoldipine assay. Using the first-order derivative spectrum, the minimum detectable amount of nitroso analogue in the presence of nisoldipine was equivalent to an impurity level of 5% and by the second-order derivative spectrum, the determination limit was equivalent to an impurity level of 2%. The degradation of nisoldipine followed within 30 days and the calculated maximal degradation rate was 1.6% per day for nisoldipine raw material, but significantly lower values of 0.19 and 0.15% per day were obtained for Nisoldin® tablets (10 and 5 mg, respectively).

Keywords: Nisoldipine; Photodegradation; Derivative UV spectrophotometry

1. Introduction

Nisoldipine ((±)3-isobutyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate) is a calcium-channel-blocking 1,4-dihydropyridine derivative, with nonidentical ester functions, which has been developed as an antihypertensive and antianginal drug [1].

The exposure of some 1,4-dihydropyridines to light leads to photodecomposition [2–6]. The chemical changes in the irradiated molecule of nisoldipine involve the oxidation of the dihydropyridine ring to a pyridine ring, and the reduction of the aromatic nitro group to a nitroso group. Depending on the source of irradiation, two photodegradation products of nisoldipine (I) are obtained (Scheme 1).

One is the nitrophenylpyridine product (III) elicited by ultraviolet light, and the other the nitrosophenylpyridine product (II) caused by daylight irradiation. An electrochemical study of nisoldipine photodegradation has been published recently [7], as well as an analysis of its analytical application in pharmaceutical forms.

Stability of nisoldipine in organic solutions was studied by GC [8] and dual-wavelength UV spectrophotometric methods [9], as well as in inclusion complexes with β-cyclodextrin [10,11].

The literature data with respect to this group of drugs also concern the assay of nifedipine tablets using first-order UV spectrophotometry [12], determination of nifedipine in a mixture with atenolol by UV derivative spectrophotometry and gas chromatography [13], as well as gas chromatographic and the third-order UV derivative determination of nitrendipine and its photodegradation product [14].

* Corresponding author.

0014-827X/00/$ - see front matter
PII: S0014-827X(00)00004-5

Recently, the photochemical degradation of nisoldipine in UV light, in ethanol solutions, was investigated by means of HPLC and MS [15], but no study has been reported considering the stability and degradation of solid nisoldipine. The objective of the present work was the photodecomposition study of nisoldipine raw material and nisoldipine film-coated tablets, after exposure to daylight, by means of UV derivative spectrophotometry. The application of derivative techniques to spectroscopy is very useful when signal overlap or interference exists and it offers a powerful tool for both qualitative and quantitative analysis of mixtures in pharmaceuticals and biomedical analysis.

2. Experimental

2.1. Material and methods

2.1.1. Chemicals

Nisoldipine ((±)3-isobutyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate) and its nitroso analogue ((±)3-isobutyl-5-methyl-2,6-dimethyl-4-(2-nitrosophenyl)-pyridine-3,5-dicarboxylate), were obtained as standard substances from Promed (Prague, Czech Republic). Nisoldipine raw material and Nisoldin® film-coated tablets containing 5 and 10 mg of nisoldipine were obtained from Slaviamed (Belgrade, Yugoslavia). Methanol p.a. from E. Merck (Darmstadt, Germany) was used as solvent.

2.1.2. Apparatus

A Perkin–Elmer Lambda-15 UV–Vis spectrophotometer (San Jose, USA) equipped with 10 mm quartz cuvettes was used. The instrumental parameters were 120 nm/min scan speed, 2 nm slit width, $\Delta\lambda = 10$ nm and response (time constant) 5 s.

2.2. Assay

2.2.1. Stock solutions

Nisoldipine (0.1 mg/ml) and its nitroso analogue standard substance in methanol was used.

2.2.2. Calibration solutions

A series of six standard solutions of nisoldipine were prepared by dilution of the corresponding stock solution to obtain a concentration range of 1–10 μg/ml and were used in the first-order derivative spectrophotometric assay.

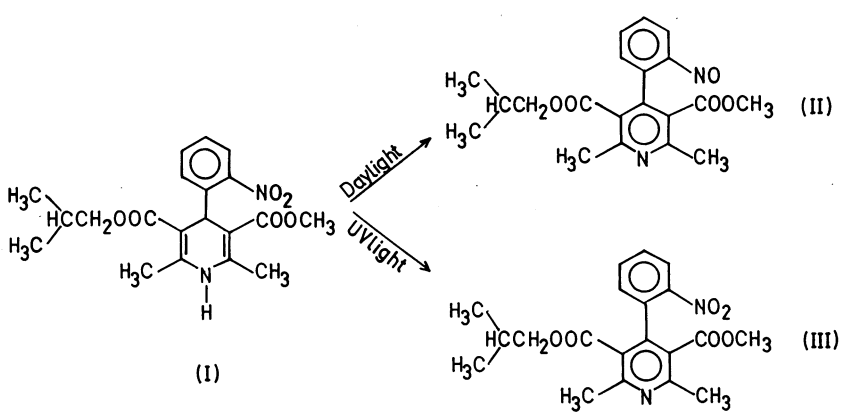
A series of six standard solutions of nitroso analogue were prepared by diluting the corresponding stock solution to obtain the concentration ranges 0.4–20 μg/ml and 0.2–5 μg/ml, which were used in the second-order and first-order derivative spectrophotometric assays, respectively.

2.2.3. Sample preparation

Nisoldipine raw material (1 g), distributed in a thin layer (< 1 mm) on a small Petri dish, was exposed to daylight for 30 days at room temperature (25°C). Nisoldin® tablets were kept in a glass container and also exposed to daylight for the same amount of time. Samples were taken from the Petri dish every 5 days and analyzed immediately. A quantity of powdered tablets containing 10 mg of nisoldipine was extracted with methanol and the final concentrations were approximately 20 and 5 μg/ml for the second-order and first-order derivative spectrophotometric assays, respectively. The same concentrations for the corresponding derivative spectra of standard nisoldipine samples were analyzed.

2.2.4. Procedure

For analysis of nisoldipine and its nitroso analogue, zero-order spectra were recorded against the solvent in the wavelength range 250–350 nm. The first-order



Scheme 1. Photodegradation products of nisoldipine (I) under ultraviolet and daylight exposure, nitrophenylpyridine (III) and nitrosophenylpyridine (II).

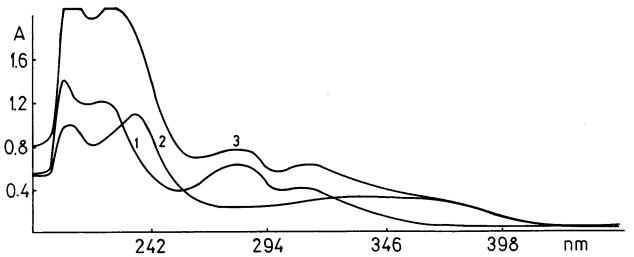


Fig. 1. Zeroth-order UV spectra of nisoldipine (20 μg/ml; curve 2); nitroso analogue (20 μg/ml; curve 1) and their binary mixture of the same concentrations (curve 3).

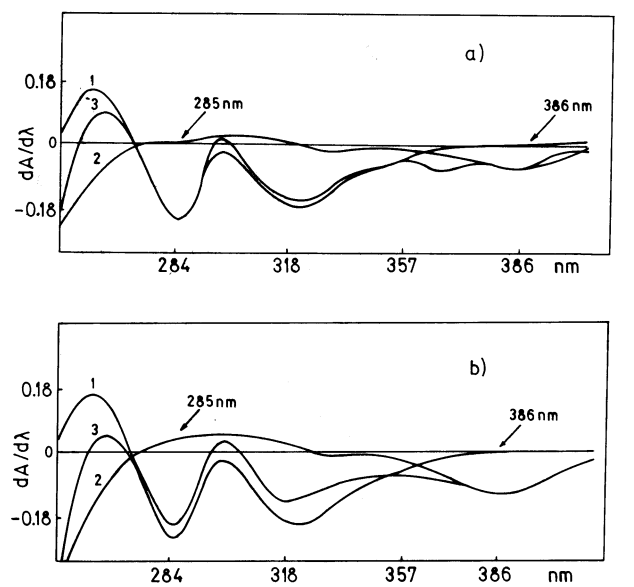


Fig. 2. First-order derivative UV spectra of: nisoldipine (curve 2); nitroso analogue (curve 1) and their binary mixture of the same concentrations (curve 3). Concentrations of nisoldipine and its nitroso analogue were 5 and 1 μg/ml and 7.5 and 1 μg/ml for (a) and (b) respectively.

derivative spectrum, obtained by analogue differentiation, is utilized for the simultaneous determination of nisoldipine and nitroso analogue using amplitudes $^1D_{386}$ and $^1D_{285}$, respectively. The corresponding amplitude $^2D_{291}$ is used for determination of the nitroso analogue. The notation for amplitude measurements was accomplished according to a more generally applicable method [16].

The limits of detection for the nitroso analogue were experimentally determined using the concentrations 0.2 and 0.4 μg/ml for the first- and second-order derivative spectra, respectively. Derivative spectra were recorded in the wavelength range 450–250 nm, for three sample solutions of each concentration, and limits of detection were obtained by measuring the corresponding average values of amplitude $^1D_{285}$ and $^2D_{291}$ in comparison with the noise signal (measured in λ range 450–400 nm).

3. Results and discussion

3.1. Spectrophotometric analysis

In the routine analysis of nisoldipine raw material, as well as in the assay of dosage forms, the conventional UV spectrophotometric method at 360 nm is used as the industrial reference method (Slaviamed, Belgrade, Yugoslavia). The structural similarity of the nitroso analogue and the parent compound leads to difficulty in the conventional UV spectrophotometric methods of analysis for this group of compounds, as their UV spectra overlap extensively, as seen for nisoldipine (curve 2) and its nitroso analogue (curve 1) in Fig. 1. This invalidates the usual compendial procedure of using absorbance measurement at a single wavelength for nitroso analogue determination, and also because nisoldipine in dosage forms is in a large excess with respect to its degradation product, nitroso analogue.

The use of derivative spectroscopy for quantitative analysis of closely related analytes requires the careful selection of the appropriate analytical bands, the appropriate derivative order and optimization of all relevant instrumental parameters. The transformation to the first-order derivative spectra of nisoldipine and its nitroso analogue (Fig. 2) indicates the possibility for the simultaneous assay of both compounds. In the wavelength range 274–290 nm the first-order derivative spectrum of nisoldipine showed no significant signal, since the zeroth-order spectrum of nisoldipine in this wavelength range (minimum of spectra) has a constant absorbance value. The effect of the first-derivative technique in discriminating against a constant nisoldipine interference for nitroso analogue determination is sufficient. For nisoldipine concentrations lower than 5 μg/ml, the spectrum was essentially zero (Fig. 2(a), curve 2). In this part of the first-order derivative spectrum, the existence of the amplitude $^1D_{285}$ of nitroso analogue was substantial (Fig. 2(a), curve 1). The value of the amplitude $^1D_{285}$ obtained for a binary mixture of nisoldipine and its nitroso analogue was the same as the corresponding $^1D_{285}$ of the standard nitroso analogue solution, confirming that the selected amplitude is due only to the nitroso analogue concentration (Fig. 2(a), curves 1 and 3). This derivative order is limited to the parent compound concentration (up to 5 μg/ml) that is presented in Fig. 2(b). For nisoldipine concentrations higher than 5 μg/ml the first-order derivative spectrum (Fig. 2(b), curve 2) shows positive values in the wavelength range 270–300 nm. Due to this, the difference for amplitude $^1D_{285}$ of the standard nitroso analogue solution and the binary mixture is significant (Fig. 2(a), curves 1 and 3). Determination of nisoldipine using a first-order derivative spectrum was considered using the amplitude $^1D_{386}$, since there was no influence of its nitroso analogue (Fig. 2). A proposed assay for nisol-

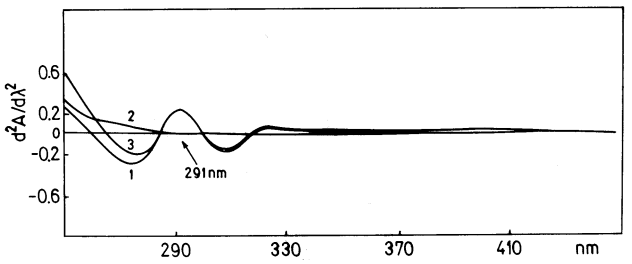


Fig. 3. Second-order derivative UV spectra of: nisoldipine (20 μg/ml; curve 2); nitroso analogue (20 μg/ml; curve 1) and their binary mixture of the same concentrations (curve 3).

dipine using the amplitude $^1D_{386}$, in comparison with the literature data [12] concerning first-order derivative UV spectrophotometry for nifedipine determination using 214 nm as detection wavelength, offers a more suitable wavelength for determination, as well as a lower concentration range compared with the obtained range for nifedipine of 5–45 μg/ml.

The least-squares regression equations for the first-order derivative spectra obtained for nisoldipine and its nitroso derivative in the concentration ranges 1–10 and 0.2–5 μg/ml, respectively, with the corresponding values for correlation coefficients are:

for nisoldipine $y = 0.021 \times -0.0013$; $r = 0.9993$

for nitroso analogue $y = 0.1399 \times +0.0009$; $r = 0.9999$

Using a nitroso analogue concentration of 0.2 μg/ml, the experimentally obtained value for the limit of detection was 0.05 μg/ml, defined as the concentration giving an amplitude signal that is three times higher than the noise signal, while a quantification limit of 0.15 μg/ml was considered as the concentration that is three times the limit of detection.

The repeatability of the proposed method was evaluated for concentrations of 10 and 5 μg/ml of nisoldipine and nitroso analogue, respectively, and the obtained values of relative standard deviations (RSD) were 0.44 and 0.62% ($n = 6$).

The effect of high concentrations of nisoldipine on the determination of the nitroso analogue was evaluated by determining the percentage recovery of the nitroso analogue in binary mixtures. The solution containing a constant nisoldipine concentration of 5 μg/ml, with no detectable amount of its nitroso analogue, was spiked with an aliquot at two concentrations of 0.25 and 0.5 μg/ml of the nitroso analogue. The percentage recoveries achieved using the amplitude $^1D_{285}$ were 98.7 and 100.1% ($n = 5$). Considering the recovery values, the minimal detectable amount of nitroso analogue in the presence of nisoldipine at a concentration of 5 μg/ml was 0.25 μg/ml (equivalent to an impurity level of 5%). Using the first-order derivative spectrum, the determination of the nitroso analogue was not feasible at an impurity level lower than 5%.

In order to determine a lower content of the nitroso analogue in the presence of nisoldipine, transformation to the second-order derivative spectra was accomplished. In the second-order derivative spectrum of the nitroso analogue, the amplitude $^2D_{291}$ is entirely independent of any nisoldipine present, confirming that this derivative order is sufficient to deconvolute the overlapping spectra of these compounds (Fig. 3). In the wavelength range 275–320 nm the second-order derivative spectrum of nisoldipine, at a higher concentration (20 μg/ml) than the one used for the first-derivative assay, is actually zero. This was established by analyzing the amplitude $^2D_{291}$ of the binary mixture with the corresponding amplitude $^2D_{291}$ of the standard nitroso analogue solution, since the same value is obtained (Fig. 3, curves 3 and 1, respectively). The amplitude $^2D_{291}$ corresponds to the peak of the long-wavelength satellite with respect to the derivative baseline zero. Since satellites in second- and also in fourth-derivative spectra are rather smaller than the actual derivative peak itself, some sensitivity may be lost, but the greater displacement of a satellite away from the centroid peak may fortuitously place it in a spectral zone clear of overlapping interferences.

The amplitude of the centroid peak at 274 nm could not be used, since as can be seen from Fig. 3 (curves 1 and 3), this amplitude is in a spectral zone of nisoldipine overlapping interference.

The least-squares regression equation for nitroso analogue determination, in the second-order derivative assay at 291 nm, in the concentration range 0.4–20 μg/ml, with the corresponding value for the correlation coefficient is $y = 0.016 \times + 0.0004$; $r = 0.9998$. The repeatability of the proposed method using $^2D_{291}$ was evaluated at a nitroso analogue concentration of 20 μg/ml, and the obtained RSD was 0.65% ($n = 6$).

Using a nitroso analogue concentration of 0.4 μg/ml, the experimentally obtained value for the limit of detection was 0.1 μg/ml, defined as the concentration giving an amplitude signal that is three times higher than the noise signal, while a quantification limit of 0.3 μg/ml was considered as the concentration that is three times the limit of detection.

Stock solutions containing a fixed nisoldipine concentration of 0.1 mg/ml, with no detectable amount of its nitroso analogue, were diluted to a working concentration of 20 μg/ml and were spiked with an aliquot of a nitroso analogue solution at two concentrations of 0.4 and 1 μg/ml (equivalent to impurity levels of 2 and 5%, respectively). The percentage recoveries for the nitroso analogue using the amplitude $^2D_{291}$ were 100.98 and 99.79%, respectively ($n = 5$). Considering the obtained recovery values, the minimal detectable amount of nitroso analogue in the presence of nisoldipine was 0.4 μg/ml (equivalent to an impurity level of 2%).

Table 1
The degradation analysis of nisoldipine raw material and commercial forms of nisoldipine

Sample	Nisoldipine raw material % degradation [a]		Nisoldin® tablet (10 mg) % degradation [a]		Nisoldin® tablet (5mg) % degradation [a]	
Days	First-order	Second-order	First-order	Second-order	First-order	Second-order
5	9.80	9.90				
10	20.00	20.05		2.10		2.10
15	25.59	26.70		2.70		2.60
20	38.50	37.75		3.60		3.50
25	42.50	42.10		4.50		4.00
30	49.90	48.89	6.00	5.80	5.00	4.50

[a] % degradation defined as nitroso analogue content expressed in %. Three replicates of each sample determination with average value of RSD (%) up to 1.1%.

3.2. Photostability studies

The first- and second-order derivative assay was applied to the analysis of commercial dosage forms of nisoldipine (Nisoldin® tablets) in comparison with nisoldipine raw material, before and after irradiation by daylight. The obtained results are presented in Table 1.

Both the first- and second-order derivative spectrophotometric methods can be used for nitroso analogue determination in nisoldipine raw material, since its content is already approximately 10% after 5 days of irradiation by daylight. Only the second-order derivative assay can be utilized for Nisoldin® tablets for the period between 5 and 30 days. The first-order derivative assay is feasible for analysis of dosage forms only after 30 days, when the nitroso analogue content was found to be 5% or higher than 5%.

Regression and correlation analysis was applied to the dependence of the nitroso analogue content (expressed in %) on time (days) of exposure to daylight. On the basis of the linear dependence (correlation coefficients $r > 0.99$) of the increase of nitroso analogue content on exposure time to daylight of nisoldipine raw material, as well of Nisoldin® tablets, the calculated maximal degradation rate in nisoldipine raw material was found to be 1.6% per day, while significantly lower and related values were obtained of 0.19% per day and 0.15% per day for 10 and 5 mg Nisoldin® tablets, respectively.

Since the whole amplitude $^2D_{291,274}$ has not been used in this assay, but only the long-wavelength satellite amplitude $^2D_{291}$, the value of the minimal detectable amount of nitroso analogue (2%) could not be lower, but it is sufficient to follow degradation kinetic where fast and significant changes are obtained.

4. Conclusions

In conclusion, it may be considered that the selectivity, linearity, accuracy and repeatability of both the first-order and second-order UV-derivative assay were satisfactory for routine analysis of nisoldipine degradation. Transformation of the broad absorption spectrum of nisoldipine to the first-order derivative spectrum offers more sensitive determination of nisoldipine using $^1D_{386}$, whereby the wavelength of the amplitude is better defined in comparison with the wavelength of the absorption maximum in zero-order spectra, which is substantial for the analysis concerning dissolution tests.

The first-order derivative assay of the nitroso analogue has the advantage for the stability analysis of nisoldipine raw material, as well as for the investigations of nisoldipine stability in organic solvents, due to the relatively fast degradation process. The first-order derivative assay for the nitroso analogue has a limitation with the parent-compound concentration (up to 5% impurity level). Using the second-order derivative spectrum ($^2D_{291}$) the determination limit of the nitroso analogue in nisoldipine was considerably lower (2% impurity level). This derivative order has an advantage over the first order for the analysis concerning the stability of dosage forms.

Daylight is an important factor in storage of the raw material, standard substance, as well as the dosage forms of nisoldipine. The film of the tablets protected nisoldipine from photodegradation, and the obtained results established that the degradation rate of the film tablets was ten times lower than that of the nisoldipine raw material.

References

[1] S. Kazda, B. Garthoff, H. Meyer, K. Schlossmann, K. Stoepel, R. Towart, W. Vater, E. Wehinger, Pharmacology of a new calcium antagonistic compound, isobutylmethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinecarboxylate (Nisoldipine, BAY-k-5552), Arzneim.-Forsch./Drug Res. 30 (1980) 2144–2162.

[2] A.L. Zanocco, L. Diaz, M. Lopez, L.J. Nunez-Vergara, J.A. Squella, Polarographic study of the photodecomposition of Nimodipine, J. Pharm. Sci. 81 (1992) 920–924.

[3] P.O. Greiner, D. Angignard, J. Cahn, High performance liquid chromatography of a new 1,4-dihydropyridine: applications to pharmacokinetic study in dogs, J. Pharm. Sci. 77 (1988) 387–389.
[4] I.A. Majeed, W.J. Murray, D.W. Newton, S. Othman, W.A. Al-turk, Spectrophotometric study of the photodecomposition kinetics of nifedipine, J. Pharm. Pharmacol. 39 (1987) 1044–1046.
[5] P. Tompe, V. Hoffmann-Fekete, E. Barczay, Studies on the photostability of nifedipine, Acta Pharm. Hung. 66 (1996) 15–19.
[6] T. Jamaoha, S. Yokoyama, T. Omote, K. Naitoh, K. Yoshida, Photochemical behavior of nifedipine derivatives and application to photosensitive polyimide, J. Photopolym. Sci. Technol. 9 (1996) 293–304.
[7] A. Alvarez-Lueje, L. Naranjo, L.J. Nunez-Vergara, J.A. Squella, Electrochemical study of nisoldipine: analytical application in pharmaceutical forms and photodegradation, J. Pharm. Biomed. Anal. 16 (1998) 853–862.
[8] J. Van Harten, M.Th.M. Lodewijks, J.W. Guyt-Scholten, P. Van Brummelen, D.D. Breimer, Gas chromatographic determination of nisoldipine and one of its metabolites in plasma, J. Chromatogr. Biomed.-Appl. 423 (1987) 327–333.
[9] Ch. Huang, Studies on photo degradation stability of nisoldipine, Zhongguo Yiyeo Gongye Zazhi 27 (1996) 26–28.
[10] J. Mielcarek, Inclusion complexes of nifedipine and other 1.4-dihydropyridine derivatives with cyclodextrins. V. The IR and X-ray diffraction study on photochemical stability of the inclusion complexes formed by nimodipine, nisoldipine and nitrendipine with β-cyclodextrin, Acta Pol. Pharm.-Drug Res. 52 (1995) 465–470.
[11] J. Mielcarek, Inclusion complexes of nifedipine and other 1.4-dihydropyridine derivatives with cyclodextrins. IV. The UV study on photochemical stability of the inclusion complexes of nisoldipine, nimodipine, nitrendipine and nicardipine with β-cyclodextrin in the solution, Acta Pol. Pharm.-Drug Res. 52 (1995) 459–463.
[12] G. Yuan, B. Zhu, N. Jin, First order derivative UV-spectrophotometry of nifedipine tablets, Zhongguo Yiyeo Gongye Zazhi 27 (1996) 171–172.
[13] M. Veronico, G. Ragno, C. Vetuschi, Simultaneous assay of atenolol and nifedipine by UV derivative spectrophotometry and gas chromatography, Spectrosc. Lett. 28 (1995) 407–415.
[14] G. Ragno, M. Veronico, C. Vetuschi, Gas chromatographic and UV derivative determination of nitrendipine and its photodegradation product, Int. J. Pharm. 99 (1993) 351–355.
[15] A. Michelitsch, J. Reiner, M. Schubert-Zsilavecz, W. Likussar, 2,2′-Bis(isobutyloxycarbonyl-5-methoxycarbonyl-2,6-dimethyl-4-pyridyl)-azobenzene-*N*,*N*′-dioxide: a new degradation product of nisoldipine by UV light, Pharmazie 50 (1995) 548–549.
[16] A.A. Fasanmade, A.F. Fell, Determination of chlorpromazine and its sulphoxide in pharmaceutical dosage forms by third-order derivative ultraviolet spectroscopy, Analyst 110 (1985) 1117–1124.