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International Journal of Pharmaceutics 233 (2002) 207–215

international journal of pharmaceutics

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The effect of ionizing radiation on some derivatives of 1,4-dihydropyridine in the solid state

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Received 30 May 2001; received in revised form 31 October 2001; accepted 1 November 2001

Abstract

The effect of gamma and beta radiation in doses between 10 and 100 kGy on physico-chemical properties of four derivatives of 1,4-dihydropyridine (nifedipine, nitrendipine, felodipine and nimodipine) in the solid state was analysed. A number of qualitative and quantitative methods such as UV, IR, TLC, GLC, DSC, EPR as well as organoleptic and gravimetric analysis were used to determine and analyse any changes resulting from irradiation. In order to determine the effectiveness of sterilization with ionizing radiation of doses from 10 to 25 kGy, various microbiological tests were used. It was established that only doses 10–20 kGy of both kinds of radiation ensure total sterilization without any degradation of physico-chemical properties of the compounds studied. For the doses 50–100 kGy a decrease in the content of the compounds, appearance of the products of their decomposition and changes in the melting point and IR spectra appeared. Felodipine (with chlorophenyl substituent) was found to be much more sensitive to ionising radiation than nifedipine, nitrendipine and nimodipine (all with nitrophenyl substituent).

Keywords: Nifedipine; Nitrendipine; Felodipine; Nimodipine; γ, β-sterilization

1. Introduction

A large number of derivatives of 1,4-dihydropyridine have pharmacological applications but the most commonly used are: nifedipine (NF), felodipine (FD), nimodipine (NM) and nitrendipine (NT). They are all calcium channel blockers and are widely used in the treatment of arterial hypertension and ischaemic heart disease (Podlewski and Chwalibogowska, 1994). The most important part of their structure is a dihydropyridine system substituted with a phenyl ring with a nitro group or (as is the case with felodipine) with two chlorine atoms. The presence of these two substituents makes these compounds very light sensitive (Marciniec and Rychcik, 1994; Marciniec and Kujawa, 1995). As the use of ionizing radiation for both sterilization and obtaining appropriate microbiological purity is increasing rapidly, it is essential to know whether the derivatives of 1,4-dihydropyridine are sensitive to this

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0378-5173/02/$ - see front matter
PII: S0378-5173(01)00938-3

type of radiation. Gamma, beta and X-ray radiation used for sterilization have ionizing proprties and are destructive towards many drugs in solution and in the solid state (Bögl, 1985; Zegota et al., 1994; Gopal et al., 1998).

In the current work we set out to test whether ionizing radiation affects physico-chemical properties of 1,4-derivatives of dihydropyridine. Four derivatives were examined (NF, FD, NT, NM). Irradiated and non-irradiated substances in the solid state were examined simultaneously using organoleptic methods (appearance, colour, smell, clarity), chromatographic methods (TLC, GLC), spectroscopic methods (UV, IR, EPR), microbiological methods (sterility tests) and others (DSC).

The results of these experiments were to answer the following question—can substances that are so sensitive to light as derivatives of 1,4-dihydropyridine be irradiated with ionizing radiation and if so—what is the effective minimum dose.

2. Materials and methods

2.1. Materials

Nifedipine (*NF*): 1,4-Dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid dimethyl ester; serial number: LOT 57H0977, obtained from Polpharma SA in Starogard Gdanski.

Nitrendipine (*NT*): 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid ethyl methyl ester; serial number: 030496.

Nimodipine (*NM*): 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedi-carboxylic acid isopropyl-2-methoxyethyl ester; serial number: 0112/95.

Felodipine (*FD*): 1,4-Dihydro-2,6-dimethyl-4-(2,3 - dichlorophenyl) - 3,5 - pyridine - dicarboxylic acid ethyl methyl ester; serial number: 9608.

NT, NM, FD were obtained from the Pharmaceutical Institute in Warsaw (Poland). All the compounds used were in accordance with USP XXIII (1996) for NF.

2.2. Irradiation with gamma and beta radiation

Approximately 0.1 g of each substance was placed in colourless jars of 3 ml volume and closed with a plastic stopper. They were irradiated with gamma radiation to 10, 20, 25, 50 or 100 kGy using a ^{60}Co gamma ray source (RChM-gamma 20). Beta irradiation was done with the help of linear electron accelerator LAE 13/9, the energy of electrons was 9.96 MeV and current intensity 6.2 μA.

2.3. Measurement of the degree of dispersion

Scanning Electron Microscope (SEM 515, Philips) micrographs confirmed that all compounds had a crystalline structure. The degree of dispersion was very close. The size of more than half of particles (65–75%) were up to 60 μm; the remaining particles were between 60 and 200 μm.

2.4. Determination of water content

Water content of all samples was determined by Karl Fischer method using Mettler Toledo, type DL 38 K. Fischer Titrater (Table 1).

Table 1
Characteristic parameters of studied compounds

Compound	Content by UV method [%]	Water content [%]	Size of particles <60 μm [%]	Impurity
Nifedipine	98.78	0.46	67.6	Accordance with USP XXIII (1996) for NF
Nitrendipine	98.83	0.26	64.3	
Nimodipine	99.36	0.56	70.2	
Felodipine	100.63	0.51	68.5	

2.5. Organoleptic analysis

The substances were examined before and after irradiation with respect to their appearance, colour, smell and clarity of the solution obtained by dissolving a particular compound in methanol. All experiments were performed in accordance with FP V Pharmacopoea Polonica (1990, p. 22–26).

2.6. Analysis by change in mass

The substances were weighted before and after irradiation on an analytical scale KERN 70 with accuracy to four decimal digits.

2.7. Thin layer chromatography (TLC)

Plates of dimensions 5.0×20.0 cm, covered with silica gel Kisegel 60 F_{254} were used. The mobile phase was benzene–methanol (6:1). The traces were set with a quartz lamp working at $\lambda = 254$ nm (Marciniec et al., 1992).

2.8. Gas–liquid chromatography (GLC)

Hewlett Packard instrument, type 5890, with a capillary column with Ultra-1 filter of dimensions 20 m × 0.2 mm was used. The column was heated from 60 to 300 °C with the speed 5 °C min^{-1}, Helium was used as a carrying gas. The thickness of the stationary phase was 0.32 μm.

2.9. Infrared spectroscopy

A KBr disc was prepared by mixing 0.75–1.00 mg of a substance with 300 mg of KBr and compressing it with Pye Unicam minipress. The spectra were recorded using a Bruker IR spectrometer in the range 650–4000 cm^{-1} with KBr as a blank.

2.10. UV spectrophotometry

The solutions were prepared by dissolving the substance in methanol to obtain concentrations between 0.05 and 0.25% w/v. The solutions were studied using UV–VIS PERKIN–ELMER Lambda 20 spectrophotometer, in 1 cm cuvettes in the range 200–400 nm, using a solvent as a blank. The results were analysed statistically (SD 0.73–1.82%).

2.11. Electron paramagnetic resonance (EPR) spectroscopy

Approximately 0.3 g of a substance was placed in a quartz capillary tube of 2 mm diameter. EPR spectra were recorded on the Bruker 6/1 EMX working at 9 GHz at temperature 293 K. For quantitative analysis a reference sample of the 10^{-4} mol l^{-1} DPPH in benzene was used.

2.12. Differential scanning calorimetry (DSC)

The measurements were performed using an DSC-200 Netzsch instrument, operating with the speed of 5 °C min^{-1}. The instrument was calibrated using tin (Sn) and indium (In). The mass of a sample was 7 mg ± 5% and the accuracy of temperature measurement was ± 0.1 °C.

2.13. Microbiological tests

Irradiated and non-irradiated samples were tested for the presence of micro-organisms using a methodology recommended by the FP V Pharmacopoea Polonica (1990, p. 113–126).

Microbiological purity of non-irradiated compounds was determined by direct inoculation onto meat infusion agar and Sabouraud's media. Samples were incubated for 72 h at 37 °C for bacteria detection and for 120 h at 22 °C for fungi detection. Sterility was checked by filtering, using a membrane filter of 0.22 and 0.45 μm pore diameter and agar-gelatin and thioglycollate media.

3. Results and discussion

In the first stage of investigation, microbiological efficiency of ionizing radiation was tested. Microbiological tests confirmed the presence of some bacteria, moulds and micrococci in unirradiated substances. However, even the minimal dose of radiation, 10 kGy, which is 2.5 times smaller

Table 2
Comparison of microbiological examination of irradiated an non-irradiated samples of 1,4-dihydropyridine derivatives to dose 10–25 kGy

Compound	Microorganisms—observed growth (kGy)			
	0	10	20	25
Nifedipine	Isolated cocci isolated moulds	No growth	No growth	No growth
Nitrendipine	Isolated bacteria	No growth	No growth	No growth
Nimodipine	Isolated bacteria	No growth	No growth	No growth
Felodipine	Isolated bacteria	No growth	No growth	No growth

than the conventional dose of 25 kGy was sufficient to eliminate all microorganisms (Table 2).

The next stage of investigation was to examination the effects of ionizing radiation on physicochemical properties of these substances. Earlier experiments confirmed the presence of free radicals in all four substances irradiated with gamma radiation to doses between 10 and 20 kGy (Taiwo et al., 1999). The current work produced similiar evidence for the presence of free radicals in samples irradiated with beta radiation to a dose of 20 kGy (Fig. 1) and also determined the concentration of free radicals for NT and NM as being 17.0 spins $g^{-1} \times 10^{16}$ and 24.8, respectively.

In all (except for NF) irradiated samples slight deepening of the colour was observed (Table 3). Other organoleptic parameters (clarity, smell, appearance) remained unaltered. A very small increase in weight (below 1%) was observed for samples irradiated to 100 kGy (Table 4). This is within experimental error.

However, statistically significant differences were observed for melting temperatures determined by DSC for substances irradiated with various doses. Samples irradiated to 50 kGy with gamma radiation showed a decrease in the melting temperature for NF by 2.1 °C and an increase for NT by 1.3 °C. When beta radiation was used the melting point for NM was decreased by 2 °C and increased for NT by 1.4 °C. Statistical analysis of the results obtained from irradiated and non-irradiated samples showed that they are equally precise (F-Snedecor test; significance level 0.95, $n = 6$ and SD is 0.16–0.19). However, as far as the average values are concerned (t-Student test) the results differ significantly. The average melting point for non-irradiated nifedipine is 174.3 °C and for irradiated one 172.2 °C.

Low doses (10–25 kGy) did not give rise to any significant changes in melting temperatures. They varied between 0.1 and 0.3 °C depending on the type of radiation and the dose (Fig. 2, Table 5). The change in the melting temperature may be due to the presence of decomposition products. In order to identify these, spectrophotometric (UV, IR) and chromatographic methods (TLC, GLC) were used.

Spectrophotometric UV analysis in the range 200–350 nm did not show any new peaks. However, there were changes in peak intensities; values of absorbances increased by 6.85% for samples irradiated with gamma radiation and up to 27.5% when irradiated with beta radiation.

Similar changes were observed for IR spectroscopy. For small doses (up to 20 kGy) changes in signal intensity, up to 6%, as well as some

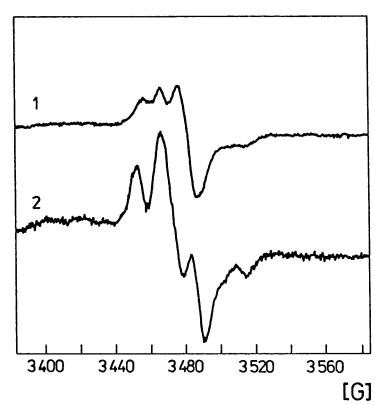

Fig. 1. EPR spectra for β-irradiated (20 kGy) nimodipine (1) and nitrendipine (2).

Table 3
Changes in colour as a result of irradiation of 1,4-dihydropiridine derivatives with gamma radiation

Compound	Colour (kGy)					
	0	10	20	25	50	100
Nifedipine	Yellow	Loss of colour intensity				
Nitrendipine	Yellow		Deepening of colour			
Nimodipine	Light cream	Deepening of colour intensity				
Felodipine	Yellow light					

Table 4
Comparison of sample weights before and after irradiation with gamma and beta radiation to 100 kGy.

Compound	Sample weigh (mg)				
	Gamma radiation			Beta radiation	
	0 kGy	100 kGy	Difference	100 kGy	Difference
Nimodypine	100.9	101.3	0.4	101.4	0.5
Nifedipine	100.2	100.8	0.6	100.6	0.4
Felodypine	101.4	101.9	0.8	101.7	0.3
Nitrendipine	103.2	104.1	0.9	101.7	0.7

shifts, between 2 and 5 cm^{-1}, were observed. Higher doses of both beta and gamma radiation gave rise to some new bands as well as disappearance or change in the shape of others (Table 6).

The TLC method showed that the starting materials were not pure and contained either two (NT, NF) or three (NM, FD) decomposition products (Table 7). The presence of impurities was also confirmed with the help of GLC (Table 8). The changes observed on the GLC chromatograms may be due to decomposition of the investigated substance as well as its impurities. They should be accompanied by the loss in the amount of the substance examined and therefore quantitative UV and GLC studies were undertaken.

Table 9 shows the changes in the content of 1,4-dihydropyridine as a result of irradiation assayed using UV method with the assumption that the content of the starting material is 100%. The largest loss (27.5%) was observed for FD irradiated with beta radiation to 100 kGy. The same dose of gamma radiation reduced the content of FD by only 5%. However, the opposite effects

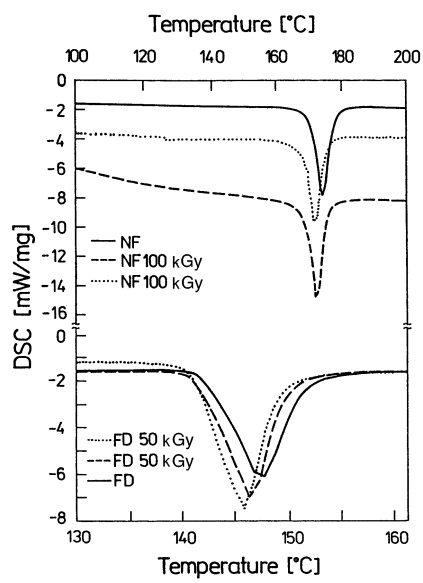

Fig. 2. DSC curves for nifedipine (NF) and felodipine (FD) before (—), after beta irradiation (----) and gamma irradiation (····).

Table 5
Comparison of melting point temperatures, determined by DSC method, before and after irradiation to doses 10–50 kGy

Compound	Melting point (°C)							
		Gamma radiation					Beta radiation	
	Literature values	0 kGy	10 kGy	20 kGy	50 kGy	Difference (°C)	50 kGy	Difference (°C)
Nimodypine	172–174	174.3	174.3	174.3	172.2	−2.1	172.9	−1.4
Nifedipine	156–160	159.1	159.8	159.8	161.1	+1.3	160.9	+1.1
Felodypine	125–128	130.7	130.7	130.7	128.8	−1.9	128.7	−2.0
Nitrendipine	142–145	147.2	147.2	147.5	145.8	−1.4	146.1	−1.1

Table 6
Changes in the IR spectrum after exposure to beta and gamma radiation

Compound	ν (cm^{-1})	Gamma radiation (kGy)				Beta radiation (kGy)	
		10	20	25	50	25	50
Nifedipine	730–860	(–) ⊗	(–) ⊗	(–)	∅ ● (–)	(–)	(–) ○ ∅
	1020–1170	(–) ⊗	(–) ⊗	∅ ⊕ ●	∅ ● (–)	(–) ∅ ○	(–)
	1470–1730	(–) ⊗ ○	(–) ⊗ ○	∅ ⊕ ○	∅ ○	(–) ●	(–) ⊗ ∅
	2980–3440	(–) ⊗	(–) ⊗	(–) ⊗	∅ ●	● ⊗	● ⊗
Nitrendipine	695–950	⊕ ⊗	⊕ ⊗	(–)	(–) ●	(–) ∅	(–)
	1170–1350	⊕ ⊗ ∅ ○	⊕ ⊗ ∅ ○	(–) ●	(–) ●	(–) ●	(–) ●
	1540–1780	⊕ ⊗	⊕ ⊗	(–) ○	(–) o ∅	(–) o ∅	(–) ● ○
	3200–3320	⊕	⊕	(–) ∅	(–)	(–) ∅	(–)
Felodipine	~850	(–) ⊗	(–) ⊗	(–) ⊗	(–) ∅ ⊗	(–)	∅
	1350–1500	(–) ⊗	(–) ⊗	(–) ⊗∅	(–) ●	⊕	(–) ∅
	1580–1750	(–)	(–)	(–)○∅	(–) ∅ ○	(–) ○	(–) ○
	3000–3400	(–)	(–)	(–)	(–)	(–)	(–)
Nimodipine	680–920	⊕	⊕	⊕	(–) ●	(–) ●	(–) ∅ ●
	1250–1380	⊕ ⊗	⊕ ⊗	⊕ ∅	(–) ⊗	(–) ∅	⊕ ⊗
	1650–1750	⊕ ∅	⊕ ∅	⊕ ○	(–)○∅	(–) ∅ ○	⊕ ○
	2980–3300	⊕	⊕	(–) ∅	(–) ∅	(–) ∅	(–) ∅

⊕ increase in band intensity; (–) decrease in band intensity; ● disappearance of a band; ∅ change in the shape of a band; ○appearance of a new band; ⊗ shift in the position of a band.

were observed for NF which showed an increase in the content by 6.85% when irradiated to 100 kGy and for FD, an increase by 5.2% when irradiated to 25 and 50 kGy. These results were then tested using GLC method (Table 10).

The results obtained from UV and GLC are significantly different. Generally, the results obtained from UV indicate a lower degree of decomposition than the results obtained from GLC (the differences are between 8 and 53%). The most likely explanation is the fact that the substance and its decomposition product may absorb at the same or nearly the same wavelength, therefore contribute to the total absorbance at that wave-

length. In the GLC method, decomposition products are separated from the original substance and therefore do not interfere with the assay. The GLC method was found to be more sensitive and specific than other methods used in this study.

Table 7
Comparison of TLC parameters for samples irradiated with gamma and beta radiation. Mobile phase: benzene–methanol 6:1. Stationary phase: Kieselgel F_{254}. Detector: UV lamp (254 nm)

Compound	Values of R_f coefficient								
	Gamma radiation (kGy)						Beta radiation (kGy)		
	0	10	20	25	50	100	25	50	100
Nifedipine					0.74		0.68	0.75	
	0.53**	0.58	0.58	0.68	0.53**	0.65	0.50**	0.58**	0.65
	0.42*	0.42*	0.42*	0.42*	0.43*	0.43*	0.44*	0.42*	0.43*
	0.25**			0.30**	0.24**		0.27**	0.23**	0.23
				0.23**					
Nitrendipine	0.50*	0.50*	0.49*	0.52*	0.52*	0.49*	0.50*	0.50*	0.49*
	0.29			0.27	0.25		0.25	0.22	0.22
	0.19**			0.23**	0.21**		0.22**	0.18**	0.18
Nimodipine	0.74			0.74	0.78	0.76	0.74	0.74	0.74
	0.53*	0.53*	0.52*	0.48*	0.50*	0.52*	0.49*	0.53*	0.50*
	0.27			0.19	0.18		0.20	0.20	0.20
	0.22								
Felodipine	0.77			0.78	0.83		0.76	0.77	
	0.50*	0.50*	0.50*	0.54*	0.56*	0.50*	0.52*	0.56*	0.50*
	0.44	0.44	0.44	0.31	0.34		0.26	0.26	
	0.35**								

* Spot for the non-irraditated substance.
** Impurities or decomposition products present in the non-irradiated sample.

Table 8
Comparison of GLC retention times for 1, 4-dihydropyridine derivatives before and after gamma irradiation

Compound	Retention times t_R (min)				
	0 kGy	10 kGy	20 kGy	25 kGy	50 kGy
Nifedipine	13.28	13.28	13.28	13.32	13.38
	12.99	13.00	12.99	12.86	12.89
Nitrendipine	14.87	14.88	14.89	14.87	14.88
	13.03			13.70	13.70
Nimodipine	17.98	17.99	17.99	17.98	17.98
	16.42	16.41	16.41	15.90	15.90
	10.03	10.00	10.01		
Felodipine	14.16	14.15	14.16	14.15	14.16
	13.32	13.32	13.33	13.33	13.36
	12.17	12.16	12.18	12.19	12.12

Table 9
Percent content of 1,4-dihydropyridine derivatives, determined by UV

Compound	λ_{max} (nm)	Content (%)							
		Gamma radiation (kGy)					Beta radiation (kGy)		
		10	20	25	50	100	25	50	100
Nifedipine	337	98.52	100.0	98.7	98.7	106.85	100.0	98.7	100.0
Nitrendipine	357	97.70	96.4	98.9	98.9	102.36	102.2	103.4	103.53
Nimodipine	360	99.48	96.1	101.3	101.3	96.49	100.0	100.0	101.75
Felodipine	362	101.2	101.2	101.2	105.2	95.00	98.7	96.2	72.5

The starting content is assumed to be 100%.

Table 10
Comparison of the average content of 1,4-dihydropyridine derivatives, determined by UV and GLC methods after irradiation with beta and gamma rays to l00 kGy

Compounds	Content (%) Gamma rays		Beta rays	
	UV	GLC	UV	GLC
Nifedipine	106.85 ± 0.96	98.04 ± 1.83	100.00 ± 0.99	80.93 ± 1.80
Nitrendipine	102.36 ± 1.30	94.38 ± 2.36	103.53 ± 1.36	83.36 ± 1.94
Nimodipine	96.49 ± 2.28	80.80 ± 2.11	101.75 ± 2.07	72.02 ± 2.03
Felodipine	95.00 ± 1.25	42.11 ± 2.68	72.50 ± 1.32	55.58 ± 2.70

4. Conclusions

On the basis of the results presented it can be said that small doses of ionizing radiation (10–25 kGy) show sufficient sterilizing properties, do not alter physico-chemical properties or cause decomposition of 1,4-dihydropyridine derivatives; however, a slight deepening of colour and a small number of free radicals was observed. Doses above 50 kGy clearly demonstrate the destructive character of ionizing radiation leading to decomposition of the original products. The resistance of the derivatives studied to gamma irradiated increases in the order: FD < NM < NT < NF, whereas to beta irradiation in the order: FD < NM < NF < NT.

The results obtained from GLC experiments indicate that beta radiation has much more destructive character than gamma radiation and therefore small doses of gamma radiation (10–15 kGy) should be used for sterilization properties.

The resistance of 1,4-dihydropirydine derivatives to low doses of gamma radiation is particularly interesting in view of their high instability to UV and VIS radiation (Thoma and Klimek, 1985).

Acknowledgements

This work was supported by the state Committee for Scientific Research (KBN) in Poland, Project no. 4PO5F 007 15.

References

Bögl, W., 1985. Radiation sterilization of pharmaceuticals-chemical changes and consequences. Radiat. Phys. Chem. 25, 425–435.

Gopal, N.G.S., Patel, K.M., Sharma, G., Bhalla, H.L., Wills, P.A., Hilmy, N., 1998. Guide for radiation of pharmaceuticals and decontamination of raw materials. Radiat. Phys.Chem. 32, 619–622.

Marciniec, B., Kujawa, E., 1995. Qualitative–Quantitative GC-MS analysis of some dihydropyridine derivatives. Chem. Anal. 40, 511–516.

Marciniec, B., Kujawa, E., Ogrodowczyk, M., 1992. Evaluation of nifedipine preparations by chromatographic-spektrophotometric methods. Pharmazie 47, 502–504.

Marciniec, B., Rychcik, W., 1994. Kinetic analysis of nifedipine photodegradation in the solid state. Pharmazie 49, 894–897.

Pharmacopoea Polonica, 5th ed., vol. I (1990) p. 22–26 and 113–126.

Podlewski, J.K., Chwalibogowska, A., 1994. Leki współczesnej terapii, Split Trading Sp. z o.o., Warszawa.

Taiwo, F.A., Patterson, L.H., Jaroszkiewicz, E., Marciniec, B., Ogrodowczyk, M., 1999. Free radicals in irradiated drugs: an EPR study. Free Rad. Res. 31, 231–235.

Thoma, K., Klimek, R., 1985. Untersuchungen zur Photoinstabilität von Nifedipin. Pharm Ind. 47, 207.

The United States Pharmacopeia, USP 23 (1996).

Zegota, H., Koprowski, M., Zegota, A., 1994. Stability of cefuroxime following gamma-irradiation in the solid state. Radiat. Phys. Chem. 43, 343–348.