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Analysis of nimodipine and its photodegradation product by derivative spectrophotometry and gas chromatography

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Abstract

A derivative spectrophotometric method for the simultaneous determination of nimodipine and its major degradation product in commercial pharmaceutical forms is proposed. The method has been defined on synthetic mixtures and compared with a new gas chromatographic procedure, as a reference method. The UV method has been used to study drug photodegradation in commercial specialities. Both methods, whose results are statistically compared, show satisfactory values of accuracy and reproducibility.

Keywords: Nimodipine; Photodegradation; Simultaneous determination; Derivative spectrophotometry; Gas chromatography

Nimodipine (NMD), isopropyl-2-methoxyethyl-1,4-dihydroxy-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate, is a dihydropyridine calcium blocker with a strong antispastic action on cerebral arteries (Haws and Heistad, 1984; Gaab et al., 1985; Kazda, 1985), used in the treatment of senile dementia and in the prophylaxis of the vascular hemicrania (Manhold, 1985; Freedman and Waters, 1987).

The drug is very sensitive to light, undergoing oxidation to its pyridine analogue (NMDP) with simultaneous transposition of the nitro group from the *meta* to *para* position (Zanocco et al., 1992). The oxidation product also represents one of the principal metabolites of nimodipine (Krol et al., 1984; Jakobsen and Mikkelsen, 1986; Rosseel et al., 1990).

In pharmaceuticals NMD has been assayed by HPLC (Jain and Jain, 1990; Shulan et al., 1991) and polarographic methods (Squella et al., 1982), but no procedure has been reported for the simultaneous determination of the drug and its photo-oxidation product.

As a continuation of the preceding works on dihydropyridine drugs (Ragno et al., 1993), this paper describes the application of UV spectrophotometry for the simultaneous assay of NMD and NMDP in bulk material and in pharmaceutical forms, using the direct measurement of specific signals in the third derivative spectrum.

A gas chromatographic method has also been developed, which, as well as in pharmaceutical

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systems, can be applied to pharmacokinetic studies on biological samples. The pyridine derivative of nitrendipine (NTDP) was used as an internal standard, since it is more stable than the starting product (Ragno et al., 1993).

The methods have been applied to the evaluation of the photodegradation rate of the NMD commercial specialities.

Nimodipine was supplied by Bayer (Milan, Italy). Nimodipine pyridine derivative was prepared by oxidation of the drug, by irradiating an ethanolic solution of 1 mg ml^{-1} with UV light (lamp of 350 nm, 50 W, at a distance of 30 cm), until the complete disappearance of the chromatographic peak of NMD (about 12 h) and purified by silica column chromatography, yielding a thick, yellow oil.

The zero- and third-order derivative spectra were recorded in the wavelength region 500–190 nm in 10 mm silica quartz cells, using a Perkin Elmer Lambda 15 spectrophotometer under the following conditions: scan speed, 2 nm s^{-1}; response (time constant), 1 s; spectral bandwidth, 1 nm; for the derivative spectra $\Delta\lambda$ was 6 nm. PECSS 4.0 package software by Perkin Elmer was used to elaborate the UV method.

A Hewlett Packard Model 5890 Series II gas chromatograph, equipped with an 'on column' injector and a flame ionization detector was used. GC was performed on a 30 m × 0.53 mm i.d. phenylmethyl silicone fused-silica wide-bore column, with a film thickness of 0.88 μm (HP5 Hewlett Packard). The oven temperature was from 230°C rising to 260°C (5°C/min); the detector temperature was 300°C. Nitrogen was used as carrier gas at a pressure of 280 kPa. Data were processed with a Hewlett Packard 3396 Series II integrator in peak-area mode.

All assay procedures were carried out in a dark room provided with a red lamp of 60 W, maintained at a distance of about 2 m.

Standard solutions: standard mixtures of NMD and NMDP for UV spectra were prepared in 95° ethanol in which NMD concentration was within the range 5.0–40.0 μg ml^{-1} and NMDP in the range 0.4–20.0 μg ml^{-1}. For GC standard solutions concentrations were within the range 0.1–2.0 and 0.05–1.0 mg ml^{-1} for NMD and NMDP, respectively. The solutions were prepared using as diluting medium an ethanolic solution with the internal standard at a concentration of 1.0 mg ml^{-1}. All injections were 1 μl. Both methods are valid at any NMD/NMDP ratio.

Synthetic preparations were made reproducing the composition of the commercial specialities. These solutions were used to establish the accuracy of the method.

Pharmaceuticals: the ethanol extracts of tablets, as well as drops and perfusion solution, were diluted to obtain an NMD nominal concentration of 25 μg ml^{-1}.

Photodegradation samples: all the photodegradation investigations were carried out in the period June–July, on clear days from 11 a.m. to 2 p.m.

An ethanol solution of NMD 30 μg ml^{-1}, in a

Table 1
Calibration graphs for the determination of NMD and NMDP

Method	Compound	Equation	r
UV	NMD	$C' = 23.408\,{}^3D_{242,233} + 0.923$ ($\pm$0.393) ($\pm$0.204)	0.9999
	NMDP	$C' = 6.904\,{}^3D_{206,199} - 15.784\,{}^3D_{242,233} + 9.498$ ($\pm$0.716) ($\pm$0.400) ($\pm$0.717)	0.9991
GLC	NMD	$C'' = 329.498\,A_{NMD}/A_{IS} - 11.852$ ($\pm$9.689) ($\pm$2.350)	0.9941
	NMDP	$C'' = 303.988\,A_{NMDP}/A_{IS} + 10.712$ ($\pm$6.104) ($\pm$3.346)	0.9961

C', concentration expressed as μg ml^{-1}; C'', concentration expressed as mg ml^{-1}. The standard deviations of the equations' coefficients are given within parentheses.

quartz cell perfectly stoppered, was analysed just after filling and then at intervals of 5 min during exposure to sunlight.

The tablet powder was evenly distributed along one of the internal surfaces of a 2 cm quartz cell, exposed to sunlight and analysed at intervals of 4 h.

30 ml of the drop solution, in a 10 cm cylindrical quartz cell, were exposed to sunlight and analysed at intervals of 3 h.

30 ml of the perfusion solution, in a 10 cm cylindrical quartz cell, were exposed to sunlight and analysed at intervals of 30 min.

The zero-order derivative spectra of nimodipine and its pyridine derivative in ethanol (Fig. 1) show a large overlap, which prevents the resolution of their mixtures by direct spectrophotometric measurements. The 354 nm absorbance maximum can be employed to measure NMD concentration when the photoproduct is below 10%, otherwise the contribution of this becomes no longer negligible.

In the third derivative spectrum of the mixture (Fig. 1), the peak-trough 242–233 nm corresponds to NMD concentration, and is not influenced by NMDP.

In contrast, the spectrum shows no specific signal for the direct determination of NMDP. Nevertheless, the peak-trough 206–199 nm results in the exact sum of the signals of the two products. In order to determine the concentration of NMDP, a multiple linear regression was defined using this signal and the above-mentioned 242–233 nm peak-trough, which allows one to correct the contribution of NMD.

The two equations are reported in Table 1.

When the method was applied on commercial tablets, the spectrum of the ethanolic solution was found to show an increase in absorbance below 210 nm, due to the absorbance contribu-

Table 2
Significance test ($p = 0.05$) for NMD and NMDP determination

Nominal		Found				Significance test			
NMD	NMDP	UV method		GC method		t-test UV [a]		t-test GC [a]	
		NMD	NMDP	NMD	NMDP	NMD	NMDP	NMD	NMDP
Standard mixtures									
30.00	9.80	30.41	9.64	28.31	10.12	$\bar{x} = 30.21$	$\bar{x} = 9.73$	$\bar{x} = 29.30$	$\bar{x} = 9.55$
		30.46	9.64	31.42	8.16	$s = 0.49$	$s = 0.13$	$s = 1.65$	$s = 0.84$
		30.62	9.72	29.54	10.26	$t = 0.99$	$t = 1.67$	$t = 0.94$	$t = 0.65$
		29.38	9.94	27.12	9.43	critical value (d.f. 4): $t = 2.78$			
		30.20	9.72	30.12	9.80				
						t-test NMD [b]		t-test NMDP [c]	
20.04	5.01	20.40	4.88	19.88	5.07	$\bar{x}_{UV} = 20.58$	$\bar{x}_{GC} = 19.97$	$\bar{x} = 0.06$	
20.04	10.02	20.77	9.86	20.53	9.75	$s = 0.15$	$s = 0.57$	$s = 0.180$	
20.04	15.03	20.60	14.84	19.42	14.78	$t = 3.16$		$t = 0.577$	
20.04	20.04	20.53	19.97	19.25	19.73	critical value (d.f. 3): $t = 3.18$			
Nimotop tablet powder									
NMD						t-test NMD [b]		F test NMD [d]	
37.52		36.92	0.02	35.89	< d.l.	$\bar{x}_{UV} = 36.58$	$\bar{x}_{GC} = 35.91$	$F = s^2_{GC}/s^2_{UV} = 1.54$	
37.50		35.71	−0.31	36.13	0.21	$s_{UV} = 0.68$	$s_{GC} = 1.32$	critical value (d.f. 3.3): $t = 9.28$	
37.51		37.30	0.30	34.21	traces	$t = 0.576$			
37.51		36.40	0.85	37.41	< d.l.	critical value (d.f. 6): $t = 2.45$			

NMD and NMDP are expressed as $\mu g\ ml^{-1}$.
[a] Comparison of the mean with a known value.
[b] Comparison of the means by UV and GC methods.
[c] Paired t-test.
[d] F test for NMD determination between UV and GLC methods.
d.f., degrees of freedom.

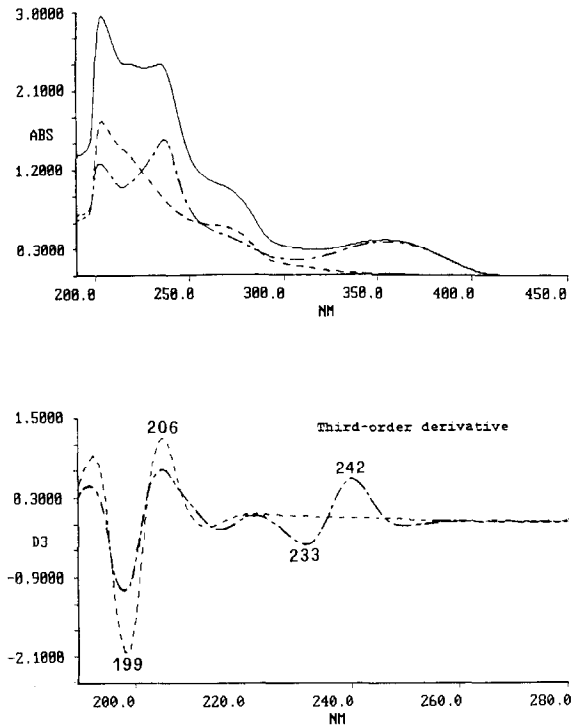

Fig. 1. Zero- and third-order derivative spectra of NMD (---) (20.04 mg ml^{-1}), NMDP (– –) (19.34 mg ml^{-1}) and their mixture (—).

tion of the excipient polyethylene glycol 4000 (PEG) which is soluble in ethanol. The effect of PEG was confirmed by preparing ethanol solutions of NMD containing the appropriate amounts of excipient. This problem can be overcome by washing the powder with water, which is very selective for PEG but leaves unextracted both analytes. The residue, after centrifugation, is treated as described above.

Gas chromatographic calibration curves for NMD and NMDP were prepared using standard solutions over the ranges reported above. Fig. 2 shows the chromatogram with an NMD concentration of 1.10 mg ml^{-1} and NMDP concentration 0.50 mg ml^{-1}. Under the reported conditions NMDP, NMD and internal standard presented retention times of 6.1 ± 0.1, 10.2 ± 0.1 and 15.0 ± 0.1 min, respectively.

The peak area ratios between analytes and internal standard were plotted against the concentration of the products, resulting in the regression relationships reported in Table 1.

The spectrophotometric method presented here was applied to the investigation of nimodipine photodegradation, in bulk as well in pharmaceutics. In particular, the half-life of the ethanol solution of NMD 30 μg ml^{-1}, as a typical concentration for UV analysis, was only 7.8 min. As regards the photodegradation rate of NMD in the commercial pharmaceutical forms, half-lives of 51.6 and 3.1 h for drops and perfusion form, respectively, were obtained, whereas the tablet powder was found to be very stable, with a decrease in NMD of 10% after 32 h. In particular, 1% degradation occurred after exposure of 40, 9 and 4 min for tablets, drops and i.v. perfusion, respectively.

The linearity between the products' concentrations and the measured values was ensured by the high values of the correlation coefficients.

Recovery and precision data were evaluated on synthetic binary mixtures of NMD and NMDP in different proportions, resulting in values of 98.4 ± 0.87 for NMD and 97.3 ± 1.77 for NMDP, applying the UV derivative method. For the GC

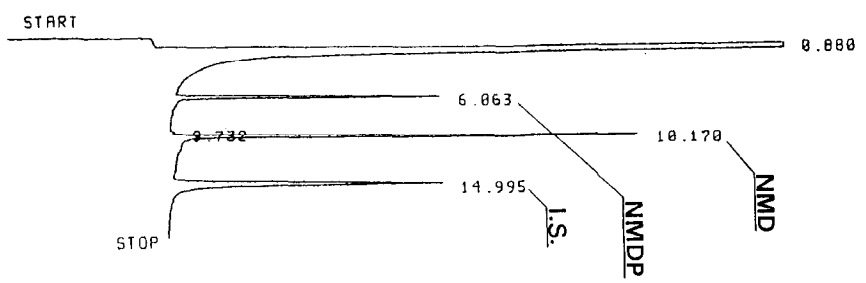

Fig. 2. GC separation of NMD (1.1 mg ml^{-1}), NMDP (0.5 mg ml^{-1}) and internal standard (1 mg ml^{-1}).

Table 3
Determination of NMD and NMDP in formulations

Speciality	UV derivative				GLC			
	NMD		NMDP		NMD		NMDP	
	Found	RSD%	Found	RSD%	Found	RSD%	Found	RSD%
Nimotop (Bayer)								
Tablets 30 mg	29.61	3.23	0.22	5.36	31.43	2.34	0.35	4.05
Drops 40 mg ml^{-1}	40.71	2.63	0.53	6.12	41.48	4.52	0.61	5.18
Perfusion 200 $\mu g\ ml^{-1}$	196.32	1.25	5.21	3.25	195.81	2.31	4.64	4.64
Periplum (Italfarmaco)								
Tablets 30 mg	30.71	1.29	0.45	4.32	31.10	3.19	0.49	6.22
Drops 40 mg ml^{-1}	39.45	2.43	0.60	3.23	40.31	3.20	0.49	3.61
Perfusion 200 $\mu g\ ml^{-1}$	194.81	1.84	3.96	3.54	197.22	4.13	4.28	5.28

method, the recovery values were 97.5 ± 2.83 and 97.5 ± 4.23 for NMD and NMDP, respectively.

Table 2 lists the results of the significance tests applied on standard solutions and pharmaceutical forms. In every test the null hypothesis is retained, the observed values of t being less than the critical values ($p = 0.05$). Therefore, no evidence of significant difference exists for the concentration means of both analytes, applying the two methods. Analogously there is no significant difference applying the F test on NMD assay with the two methods, at the 5% level.

The methods have been applied to the analysis of NMD commercial forms. The results are summarized in Table 3.

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