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Gas chromatographic and UV derivative determination of nitrendipine and its photodegradation product [1]

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Summary

A rapid and direct analytical UV method, based on third derivative spectra, and a gas chromatographic assay, for the simultaneous determination of nitrendipine and its photodegradation product, are proposed. Both procedures, showing satisfactory values of accuracy and precision, can be employed to measure the drug and its impurity in pharmaceuticals.

Nitrendipine (Ntd), 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid ethyl methyl ester, is a calcium-channel blocker with vasodilatory properties, present in several commercial preparations administered in the treatment of hypertension.

On exposure to light, the drug in the solid state as well as in solution decomposes to give the dehydrogenated pyridine derivative (NtdOx) which is also its main metabolite.

The determination of nitrendipine, performed using polarographic techniques in commercial preparations (Squella et al., 1988) or for photodecomposition studies (Squella et al., 1990), has been reported in the literature. Liquid chromatographic UV detection (Janis et al., 1983; Raemsch et al., 1984) and ECD-gas chromatographic procedures involving packed (Kann et al., 1984; Raemsch et al., 1984) or capillary (Soons and Breimer, 1988) columns have also been described. Other GC methods for analysis of the drug and its metabolites in biological fluids use mass detection (Beck and Ryman, 1985; Fischer et al., 1986). Radiochemical methods (Goula et al., 1983; Janis et al., 1983; Lee et al., 1983) which were unable to evaluate the pyridine derivative and radioimmunoassay procedures which lacked specificity (Campbell et al., 1986; Thayer et al., 1986) have also been reported.

The present note describes a rapid and accurate UV method, using third derivative spectra,

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for the simultaneous determination of Ntd and its photooxidation product in specialities and bulk material.

To confirm the spectrophotometric results, a gas chromatographic procedure has been also defined, employing the most current apparatus, which is suitable as a routine analysis for the simultaneous determination of both substances in pharmaceuticals or biological samples.

Derivative spectra were recorded over the wavelength range 350–190 nm in 10 mm silica quartz cells using a Perkin-Elmer Lambda 15 spectrophotometer under the following instrumental conditions: scan speed, 2 nm s^{-1}; response (time constant), 1 s; spectral bandwidth, 1 nm; $\Delta\lambda$, 6 nm. PECSS 3.2 software was used to manipulate the displayed curves.

Gas chromatography was performed using a Hewlett-Packard Model 5890 Series II gas chromatograph, equipped with an on-column injector, a flame ionization detector, and a fused silica wide-bore column (HP1-5 m × 0.53 mm, 0.23 μm film thickness). Operating temperatures were: injector, 260°C; detector, 350°C; oven, 120°C rising to 240°C at 10°C min^{-1}. The carrier gas was nitrogen at a flow rate of 25 ml min^{-1}. Injection volume was 1 μl.

Under these conditions, the internal standard aminophylline, NtdOx and Ntd had retention times 6.3 ± 0.1, 9.3 ± 0.1 and 11.6 ± 0.1 min, respectively. Data were processed with a Hewlett-Packard 3396 Series II integrator in peak-area mode.

Aminophylline (99% pure) was purchased from Aldrich. Ntd was obtained by acetone extraction from commercial tablets and crystallized twice using ethanol. NtdOx was obtained by degradation of Ntd. For this purpose, a methanolic solution of 1 mg ml^{-1} concentration was irradiated with a UV lamp (350 nm, 50 W, at a distance of 30 cm) until the chromatographic peak of Ntd had disappeared (about 6 h). The alcoholic solution was concentrated and the residual product subjected to column chromatography on silica gel using ethyl acetate/hexane 4:6 (v/v) as an eluent. The product fraction was evaporated to give an orange substance. Purity of the product was confirmed by gas chromatographic analysis (Ntd/NtdOx less than 0.1%, Ntd being undetectable in a solution of 0.1 mg ml^{-1}), NMR and mass spectroscopy.

The preparation and analysis of samples were performed in artificial light from a red lamp of 60 W, kept at a distance of 2 m. Standard solutions were prepared in ethanol with the concentration of Ntd being within the range 5–60 μg ml^{-1} and with NtdOx varying between 0.25 and 22 μg ml^{-1}. The Ntd/NtdOx ratio ranged from 1 to 120.

Similarly, GC standard solutions were pre-

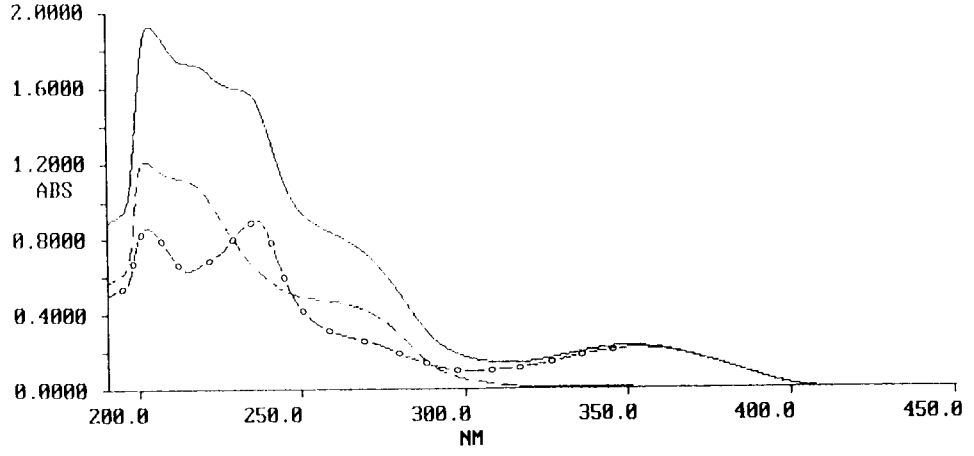

Fig. 1. Absorption spectra of Ntd (○———○) (20.64 μg ml^{-1}), NtdOx (— — —) (18.34 μg ml^{-1}) and of their mixture 1:1 (continuous line).

TABLE 1

Correlation parameters

Peak ratio	Intercept	Correlation coefficient	Standard error
Ntd/aminophylline	−0.374	0.9997	0.277
NtdOx/aminophylline	−0.0875	0.9985	0.103

pared with the same concentrations of Ntd and NtdOx, using ethanol containing 20 μg ml^{-1} of aminophylline as solvent.

Correlation equations of the amounts vs peak area ratio between analyte and internal standard were defined (Table 1).

The method was applied to pharmaceuticals. Five tablets were weighed and reduced to a fine powder. An amount corresponding to the average of one tablet, accurately weighed, was transferred to a 50 ml volumetric flask, stirred with ethanol and made up to volume with the same solvent. 1 ml of the suspension was transferred to a 10 ml volumetric flask and diluted to volume with ethanol.

This method was analogously applied to laboratory mixtures. These were prepared in order to obtain an Ntd/NtdOx ratio within the range 1–100 in the presence of the following excipients, employed in the usual amounts in pharmaceutical formulations: starch, magnesium stearate, polyvinylpyrrolidone, talc, stearic acid and stearyl alcohol.

Aliquots of these mixtures were treated with ethanol to yield concentrations ranging from 5 to 60 μg ml^{-1} for Ntd. These solutions were used to establish the accuracy of the method.

The zeroth derivative spectrum (Fig. 1) does not permit evaluation of the presence of the degradation product, whereas the maximum at 354 nm allows one to determine Ntd satisfactorily (recovery 98%; SD 2.5) only if the NtdOx level is no greater than 10%.

In contrast, the derivative spectrum shows the 241–233 nm peak-trough whose amplitude value is due only to the Ntd concentration, the contribution of NtdOx being negligible at this wavelength, whereas no specific signal for the direct evaluation of the degradation product was observed. Therefore, the NtdOx concentration was determined by multiple linear regression analysis using the amplitude of the 206–199 nm peak-trough which is influenced by both substances.

This is readily verified on inspection of Fig. 2 which displays the overlapping spectra, recorded at 1-h intervals, of an Ntd solution (EtOH 1.5 mg/ml) which had been exposed to sunlight for increasing periods of time.

In fact, the peak-trough at 241–233 nm, used for Ntd determination, undergoes a decrease until its complete disappearance, whereas the

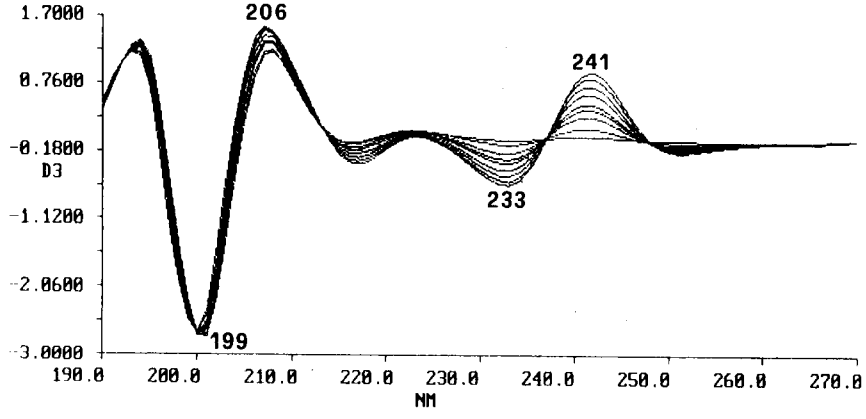

Fig. 2. Third derivative spectra of Ntd recorded immediately after preparation (60 μg ml^{-1}) and at intervals of 1 h during exposure to daylight.

TABLE 2

Analysis of commercial forms and laboratory mixtures

Sample	Nominal		UV			GLC			
	Ntd	NtdOX	Ntd	RSD%	NtdOx	RSD%	Ntd	RSD%	NtdOx	RSD%
Laboratory mixtures										
1	40.92	5.75	39.27	0.36	5.24	0.83	39.31	0.39	4.89	1.03
2	40.92	2.87	41.92	0.51	2.67	0.75	41.82	0.52	3.12	0.86
3	61.38	11.50	58.29	0.56	11.91	2.41	57.89	0.42	11.27	1.12
4	61.38	1.15	56.83	0.73	1.38	0.31	62.12	0.43	1.10	0.43
5	61.38	0.58	59.85	0.69	0.63	0.12	57.49	0.91	0.49	0.24
Pharmaceuticals										
Baypress	25		24.63	0.31	0.25	3.25	24.91	0.45	0.32	4.12
Deiten	25		25.41	0.23	0.33	2.89	24.82	0.61	0.46	4.46
Lusopress	25		24.19	0.43	0.12	4.12	25.32	0.73	0.25	3.89
Bayotensin	25		24.42	0.36	0.45	3.63	24.11	0.56	0.39	4.26

peak-trough at 206–199 nm remains almost constant, since the relative signal of Ntd, as it disappears, is substituted by that of NtdOx.

Verification of the rapid rate of degradation ($t_{1/2} = 4.8$ h) requires conditions of subdued light during every step of sample manipulation.

The Ntd and NtdOx contents were evaluated from the following linear regression equations: $[\text{Ntd}] = 37.567[{}^3D_{241,233}] - 0.674$ ($r = 0.9997$) and $[\text{NtdOx}] = 19.065[{}^3D_{206,199}] - 50.079[{}^3D_{241,233}] + 7.615$ ($r = 0.9991$) where [Ntd] and [NtdOx] are expressed as μg ml^{-1}.

The derivative method was applied to the analysis of commercial forms and laboratory mixtures. The results are summarized in Table 2.

The linearity between the measured values and the drug concentrations, for 30 real points in the reported ranges, was ensured by the high values of the correlations coefficients which were never less than 0.999. The data obtained on the recovery, applying the UV derivative method, as estimated for laboratory mixtures, were 98.42 ± 2.63% for Ntd and 102.27 ± 5.60% for NtdOx, expressed as percent of the nominal amount.

As regards the GC method, the recovery values were 97.49 ± 3.54 and 96.38 ± 6.00%, for Ntd and NtdOx, respectively. All values are means of 10 determinations with precision values expressed as SD%. These results are in good agreement with the claimed values and with those obtained by the GC method.

The GC determination limits amounted to 0.10 and 0.15 ppm for Ntd and NtdOx, respectively. The UV sensitivity for NtdOx/Ntd was 0.5%.

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