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Photodegradation monitoring of amlodipine by derivative spectrophotometry

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Abstract

A derivative spectrophotometric method for the simultaneous determination of amlodipine and its pyridine photodegradation product has been developed. The analytes concentrations were linearly correlated with spectral measurements in the 3rd order UV derivative spectrum through equations obtained by simple and multiple regression analysis. The recovery values were estimated to range from 95 to 99% and the quantitation limit of the photoproduct was found to be equivalent to an impurity level of 1%, with respect to the content of amlodipine. The method could usefully be applied to routine quality control of pharmaceutical formulations containing amlodipine.

Keywords: Amlodipine; Photodegradation; 1,4-dihydropyridine derivative; Derivative spectrophotometry

1. Introduction

Amlodipine, *R*,*S*-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (AML), is a potent, long-lasting calcium antagonist agent, widely used against hypertension and angina [1], belonging to the generally termed '1,4-dihydropyridines' class. With a feature common to all compounds of the class, the drug undergoes oxidation when exposed to light [2–4], so generating the pyridine analogue (AMLOX) (Fig. 1).

In the present study, the photo-oxidation of AML was confirmed by exposure of the drug to the light. The photodegradation product was then isolated and its structure was confirmed by MS analysis. Metabolism studies have also indicated the pyridine derivative as the major metabolite of the drug [1–5].

Due to the photosensitivity, accurate precautions are required in handling the drug during manufacturing, therapeutic and analytical trials. Amlodipine has been quantitatively assayed in biological fluids by HPLC [6] and GC [7]. Determination of the drug in pharmaceuticals dosage forms includes spectrophotometric [8–10] and chromatographic techniques [11–16] as well. However, such methods resulted only suitable for the determination of the drug, whereas any useful

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procedure for the analysis of its photodegradation product was not yet available.

Accordingly, a rapid and accurate UV derivative method for the simultaneous analysis of AML and AMLOX in raw material and pharmaceutical formulations is herein reported. The procedure was performed directly on the matrix suspension, adopting as quick as possible operating conditions, in order to minimize light exposure.

2. Experimental

To minimize the amlodipine photodegradation, all laboratory procedures were carried out under the illumination of a red lamp (60 W), kept at a distance of about 2 m.

2.1. Chemicals

Amlodipine pure powder was kindly provided by Pfizer (Rome, Italy).

The isolation of AMLOX was performed by direct oxidation of AML, by irradiating a 2 mg ml^{-1} hexane suspension of the drug with an UV lamp (280–360 nm, 30 W, at a distance of 30 cm) for 80 h. The solid, turned yellow, was filtered and washed with pure hexane. The purity and identity of the photoproduct was confirmed by gas–mass chromatographic analysis. The mass spectrum was overlapped with that reported in literature with a molecular peak m/z 407 [1,14].

Ethanol and hexane, of analytical reagent grade, were purchased by C.Erba (Italy). Norvasc (Pfizer, Italy), Monopina (Bioindustria, Italy) and Antacal (Ellekappa, Italy) pharmaceuticals were obtained commercially.

2.2. Instrumentation

The zero and third order derivative spectra were recorded in the wavelength region 190–400 nm in 10 mm silica quartz cells, using a Perkin–Elmer Lambda 15 spectrophotometer. The instrumental parameters, optimized for the third-order derivative spectrum, were, scan rate, 1 nm s^{-1}; time response, 1 s; spectral bandwidth, 1 nm; $\Delta\lambda$, 6 nm. UV WinLab 2.70.01. package software by Perkin–Elmer was used to elaborate the spectral data.

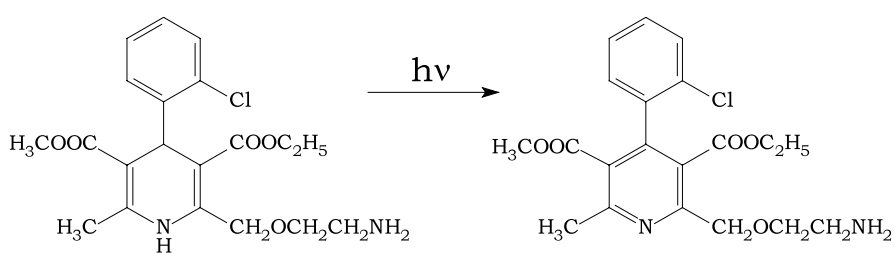
For the artificial light irradiation, a lamp whit emission between 280 and 360 nm; 30 W, was used (Sankyo Denki, Japan).

2.3. Standard solutions

Standard mixtures in ethanol 95° were prepared and used to set up the calibration curves. AML concentration was within the range 5.0–50.0 μg ml^{-1} and AMLOX in the range 0.2–50.0 μg ml^{-1}. AMLOX percentage value was between 0 and 100.

2.4. Sample solutions

Laboratory mixtures were made in order to obtain AML and AMLOX concentrations within the ranges reported in 'Standard solutions' and the ratio AML/AMLOX within the range 1–200.



AML AMLOX

Fig. 1. Formulas.

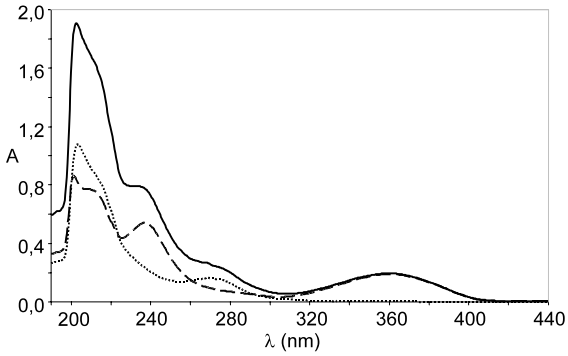


Fig. 2. Zero-order derivative spectra of AML (28.12 μg ml^{-1}) (– –), AMLOX (24.46 μg ml^{-1}) (·····) and their mixture 1:1 (—).

The following excipients were added in usual amounts used into pharmaceutical formulations, starch; magnesium stearate; talc and stearic acid. These suspensions were used to establish the accuracy of the method.

For the pharmaceuticals analysis, five tablets were weighed and reduced to a fine powder. An amount corresponding to the average of one tablet was accurately weighed, stirred and made up to a volume of 50 ml with ethanol. One ml of this suspension was then diluted to 10 ml with ethanol and analysed without filtration.

2.5. Photodegradation

The exposition to natural light was carried out in the period June–July, constantly exposing the samples from 09:00 h to 17:00 h in clear days. The irradiation with artificial light was performed by exposure to a 280–360 nm UV lamp (30 W, at a distance of 30 cm).

AML raw material was distributed in a thin layer, and analysed at various intervals during exposure. For this aim 10 mg of powder, accurately weighed, were diluted with ethanol to obtain a concentration of about 20 μg ml^{-1}.

As regards to the pharmaceutical forms, ten tablets were exposed to both natural and artificial light and analysed at different times, as reported in Section 2.4. The procedure was replicated on tablets protected by packaging materials.

3. Results and discussion

3.1. Derivative spectrophotometric analysis

The absorption spectra of amlodipine and its pyridine derivative in ethanol (Fig. 2) overlapped closely, preventing the analysis of their mixtures by direct measurements of absorbance signals. The AMLOX spectrum is completely overlapped by the AML spectrum, whereas the 360 nm absorption value for AML determination is significantly influenced by AMLOX when this is over 5%.

Therefore, the derivative spectra (first to fourth) of each of the two products and of their binary mixtures were tested. The application of derivative techniques to spectroscopy offers a powerful tool for a better resolution enhancement, when signal overlap or interference exists.Moreover, this technique appeared very useful since a short as possible assay time is necessary to be maintained, because of AML photodegradability.

In fact, being the derivative spectra not influenced by the background absorption due to the turbidity baseline, the spectral measurements could be performed directly on the turbid sample obtained by dissolving and diluting the drug tablets.

The goal of the procedure was the simultaneous determination of both the products in the same derivative order spectrum. Only the third derivative spectrum was found to provide a better resolution of overlapping absorption bands (Fig. 3). AML concentration was demonstrated to be proportional to the signal at 243 nm and not influenced by AMLOX, the absorption of the latter being negligible at that wavelength. A calibration graph was obtained by applying a least squares linear regression to the derivative amplitudes against the increasing concentrations of pure AML.

On the contrary, no specific signal directly correlated with AMLOX concentration was observed. Nevertheless, both products present in the same derivative spectrum a signal at 229 nm, which result exactly added in the mixture spectra. A multiple linear regression was derived using this

signal and the before mentioned signal $^3D_{243}$, which represents the AML contribute, versus AMLOX concentration.

In order to verify the possible AML photodegradation during UV scanning, samples of AML standard solutions were scanned from 200 to 400 nm for five consecutive times. No appreciable changes on the zero and third derivative spectra between the first and the fifth record were detected, demonstrating clearly that in the time of UV recording the photodegradation which occurs was irrelevant.

3.2. Validation

Table 1 shows the regression coefficients and linearity ranges of the calibration curves for the determinations of AML and AMLOX. Although in the routine analysis of AML products the samples present generally a low content of AMLOX, these equations were demonstrated to be able to determine both the products at any value of AML/AMLOX ratio.

To estimate the absence of errors due to the interference of AMLOX on the AML equation, a t-test on the intercept was performed assaying solutions of pure AML. The experimental t was carried out by the expression $t = b/S_b$, where b is the calculated intercept value and S_b its standard deviation. The t value (2.02) resulted less than the t critical value ($t = 2.36$, d.f. 7) at the significance level of 0.05.

The limits of detection and quantitation of AMLOX were experimentally determined. For this aim a series of standard binary solutions with 40.00 μg ml^{-1} AML and AMLOX concentration

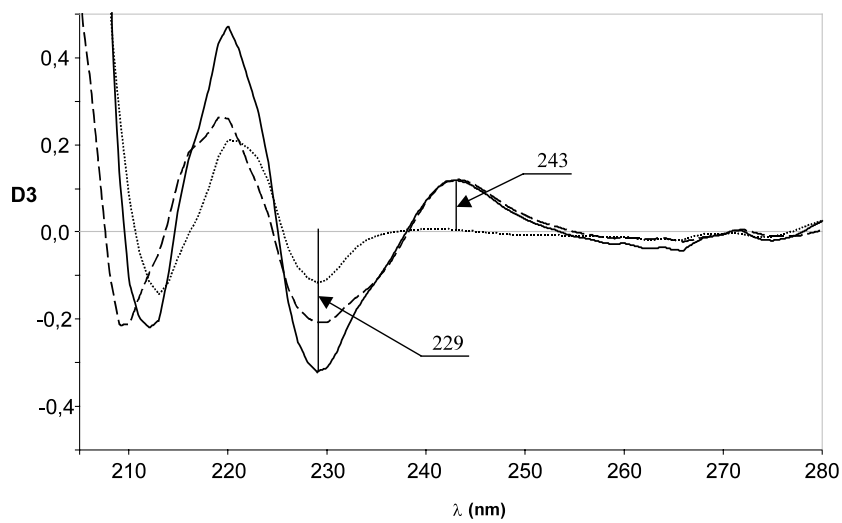


Fig. 3. Third-order derivative spectra of AML (28.12 μg ml^{-1}) (– –), AMLOX (24.46 μg ml^{-1}) (·····) and their mixture 1:1 (—).

Table 1
Calibration graphs for AML and AMLOX assay by derivative spectrophotometric method

Signal	Equation	Correlation coefficient	Concentration range (μg ml^{-1})
$^3D_{243}$ (λ_1)	AML = 174.882 λ_1 − 0.168 (± 4.516) (± 0.029)	0.9997	5–50
$^3D_{243}$ (λ_1), $^3D_{229}$ (λ_2)	AMLOX = 178.832λ_2 − 302.918λ_1 − 0.375 (± 3.689) (± 7.562) (± 0.074)	0.9994	0.2–5

The S.D. for the equations' equations are given within parentheses.

Table 2
Analysis of laboratory samples and pharmaceuticals

Sample	Nominal		Found					
	AML	AMLOX	AML	Recovery	RSD	AMLOX	Recovery	R.S.D.
Laboratory solutions (μg ml^{-1})								
1	20.12	2.21	19.60	97.41	3.47	2.14	96.92	3.27
2	30.19	5.52	29.98	99.33	1.90	5.55	100.54	0.54
3	40.25	0.55	39.82	98.94	1.83	0.53	96.01	9.43
4	50.31	1.10	49.96	99.30	1.38	1.04	94.20	6.73
5	50.31	0.55	50.22	99.81	1.61	0.58	105.07	6.90
Pharmaceuticals (mg)								
Norvasc	10.00	–	9.77	97.70	1.54	0.15	–	6.67
Monopina	10.00	–	9.87	98.70	2.33	0.11	–	9.09
Antacal	10.00	–	9.70	97.00	1.86	0.18	–	5.56

varying from 0 to 2.00 μg ml^{-1} were analysed. The limit of detection for AMLOX resulted to be 0.15 μg ml^{-1}, as the analyte concentration giving a signal equal to three times the standard deviation of the blank signal $^3D_{229}$ (R.S.D. = 1.12; $n = 5$; calculated on a 1 μg ml^{-1} AMLOX standard solution). The limit of quantitation, as the concentration value equal to three times the detection limit, resulted to be 0.45 μg ml^{-1}, equivalent to an impurity level of almost 1%.

Accuracy and precision data, were carried out on synthetic binary mixtures of AML and AMLOX in different ratio values, obtaining the results shown in Table 2. Recovery and relative standard deviation (R.S.D.) data resulted in values of 98.96 ± 2.04% for AML and 98.55 ± 5.37% for AMLOX. All values were means of six determinations. A *t*-test on data from laboratory mixtures was performed in order to verify whether the difference between the true value and the experimental mean was significant. The *t* calculated resulted in all cases less than the critical value ($t = 2.57$; d.f. 5) at the significance level of 0.05, so the 'null hypothesis' was retained.

The method was applied to the analysis of AML commercial specialties. The assay results, shown in Table 2, are in excellent agreement with the drug declared amount. The AMLOX found in some specialties resulted to be not more than 1.7%. The reproducibility of the results on commercial specialties was carried out on units with different number lot. For each trade name three units were analysed, performing the assay on four tablets for each unit. The unit means were compared by one-way ANOVA to test whether they differed significantly. The results are summarized in Table 3. In every case the null hypothesis was retained as the calculated value of *F* (between sample variation/within sample variation) resulted smaller than the critical value ($F_{2,9} = 4.256$; $P = 0.05$).

3.3. Photostability

The proposed method has been applied to study the effects of natural and artificial light on raw material and drug dosage forms, either protected or not by packaging materials.

The content of AMLOX in raw material was found to be approximately 10% after 8 and 5 h, under daylight and artificial light, respectively. The pharmaceutical tablets resulted sufficiently stable, with a decrease of AML title of 10% after 46 h of natural light exposure and 30 h under artificial light. On the contrary, the tablets resulted well protected from the photodegradation process when enwrapped by the packaging material.

Table 3
Analysis of variance (one-way ANOVA) on AMLOX found in pharmaceutical formulations

	Norvasc			Monopina			Antacal		
Tablet	Unit 1	Unit 2	Unit 3	Unit 1	Unit 2	Unit 3	Unit 1	Unit 2	Unit 3
1	0.96	1.23	1.03	1.54	1.21	1.46	1.46	1.82	1.54
2	0.91	1.05	1.24	1.27	1.43	1.58	1.74	1.75	1.64
3	1.05	0.96	1.11	1.35	1.25	1.42	1.58	1.69	1.62
4	1.10	1.25	0.96	1.34	1.35	1.52	1.65	1.70	1.50
Overall mean			1.07			1.39			1.64
Between-sample variation			0.0144			0.035			0.031
Within-sample variation			0.0139			0.009			0.0073
$F_{2,\ 9}$			1.037			3.755			4.190

The content is expressed as percentage value.

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