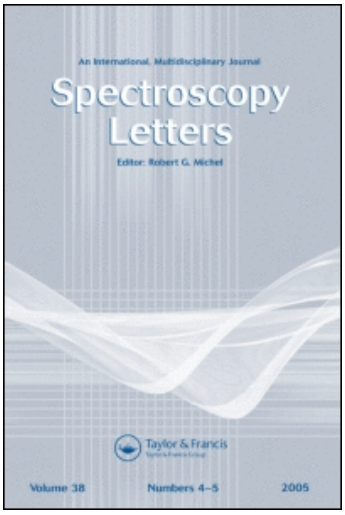

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Simultaneous Assay of Atenolol and Nifedipine by UV Derivative Spectrophotometry and Gaschromatography

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SIMULTANEOUS ASSAY OF ATENOLOL AND NIFEDIPINE BY UV DERIVATIVE SPECTROPHOTOMETRY AND GASCHROMATOGRAPHY

KEYWORDS

Atenolol; Nifedipine; Pharmaceutics; Simultaneous determination; Gaschromatography; UV Derivative Spectrophotometry;.

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ABSTRACT

A rapid, simple, and accurate UV derivative method for the simultaneous determination of atenolol and nifedipine in dosage forms was developed. A new gaschromatographic procedure was also defined to convalide the spectrophotometric procedure as an alternative method. Statistical analysis of the results on data from laboratory solutions confirms a good accuracy and precision of both the methods.

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INTRODUCTION

Atenolol (AT), 4-(2-hydroxy-3-isopropylamminopropoxy)phenyl acetamide, is a cardioselective β-blocker with sympathomimetic activity and membrane stabilizing properties [1].

Nifedipine (NF) is a calcium channel blocker commonly used as an arterial vasodilator in the management of angina and various cardiovascular diseases [2,3]. Chemically NF is the dimethyl-1,4-dihydro-2,6-dimethyl-4-(2nitrophenyl)pyridine-3,5-dicarboxylate.

Several methods, including colorimetry [4], spectrophotometry [5], GLC [6], HPLC [7,8] and NMR spectroscopy [9], have been described for determination of atenolol, as raw material, for determining the purity grade, as in tablets .

In pharmaceutical formulations, nifedipine has been determined by HPLC [10], gaschromatographic [11-13], colorimetric [14-16] and polarographic methods [17].

The combined oral formulation of the two drugs has been found to be more effective than either drug administered alone in the treatment of the hypertension. Several commercial specialities (capsules) contain the two drugs in association, nevertheless only a GLC [18] and a HPLC [19] methods have been reported for their simultaneous determination. Both methods result time consuming, besides the GLC procedure suffers from low sensitivity.

The present paper describes a simple, rapid, sensitive, and accurate UV derivative spectrophotometric method for the simultaneous determination of the two drugs in commercial preparations. The analysis is directly performed on a capsule solution, since derivative spectroscopy allows one to eliminate broad absorption bands resulting from turbidity and matrix interference.

An unpublished gaschromatographic procedure was also defined to confirm the spectrophotometric results, suitable also as alternative method.

EXPERIMENTAL

Apparatus and conditions

Spectrophotometry: absorption and derivative spectra were recorded over the wavelength range 190 - 400 nm in 10 mm silica quartz cells, using a Perkin Elmer "Lambda 15" spectrophotometer under the following conditions: scan speed 60 nm/s; time response 1 s; spectral bandwidth 1 nm; $\Delta\lambda$ 4 nm.

Gaschromatography: a Hewlett Packard "5890 Series II" gaschromatograph, equipped with a flame ionization detector was used. GC was performed on a 30 m x 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 for two minutes to 270°C (8°C/min); the detector temperature was 300°C. Nitrogen was used as carrier gas at a pressure of 180 Kpa. Data were processed with a Hewlett Packard "3396 Series II" integrator in peak-area mode.

Materials

Pharmaceutical specialities analysed were: Mixer (Biomedica Foscama, Italy) and Niften (ICI Pharma, Italy), as capsules containing 50 mg of AT and 20 mg of NF.

Atenolol and internal standard aminophylline were purchased by Sigma (U.S.A.); nifedipine was gently supplied by Bayer (Milan, Italy).

All the solvents were of analytical grade and ethanol of spectrophotometric grade.

Laboratory precautions

To minimize the nifedipine photodegradation, all studies were carried out under the illumination of an artificial light from a 60 Watt red lamp, kept at a distance of 2 meters, in the minimum possible time.

Standard solutions

Spectrophotometry: standard mixtures in ethanol were prepared with AT and NF concentration ranging from 5 to 70 μg/mL and from 4 to 50 μg/mL, respectively. AT/NF ratio was between 0.6 and 12.5 (ratio in capsules 2.5).

Gaschromatography: standard mixtures in ethanol were prepared with AT and NF ranging between 0.1 - 1.0 mg/mL for both analytes, containing, as internal standard, aminophylline at 1 mg/mL. All injections were 1 μL.

Twenty standard solutions for both methods were used to obtain the reported regression equations.

Sample solutions

Pharmaceuticals: the content of five capsules was powdered and an aliquot containing a nominal value of one capsule was accurately weighed, solubilized in ethanol and diluted to give a solution with a nominal content of AT 25 μg/mL and NF 10 μg/mL.

Laboratory mixtures: powder preparations reproducing the composition of the commercial specialities were prepared in order to obtain drug ratios AT/NF within the range 0.5-6.0.

The results obtained from the analysis of these solutions were used to establish the accuracy of the methods.

RESULTS AND DISCUSSION

Fig. 1 shows the zero-order ultraviolet spectra of AT and NF ethanolic solutions at similar concentration and of their 1:1 mixture. The maximum in the zone 300 - 400 nm, due to NF, is very flat and not useful for the drug determination. In fact analysis of NF standard solutions, by using this maximum, provided unaccurated results.

In the wavelength region between 200-300 nm the spectra of the two products clearly display considerable overlap, therefore the mixture absorbance spectrum is of no use for analytical purpose.

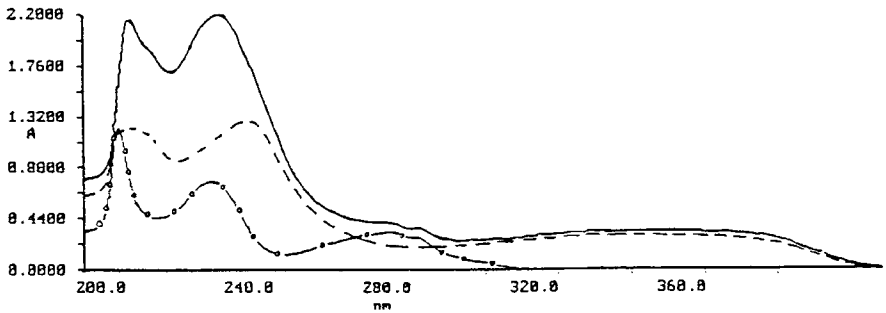

Fig.1 *Absorption spectra of NF (- - -) 20.12 μg/mL, AT (-o-o-) 20.46 μg/mL and of their mixture 1:1 (——).*

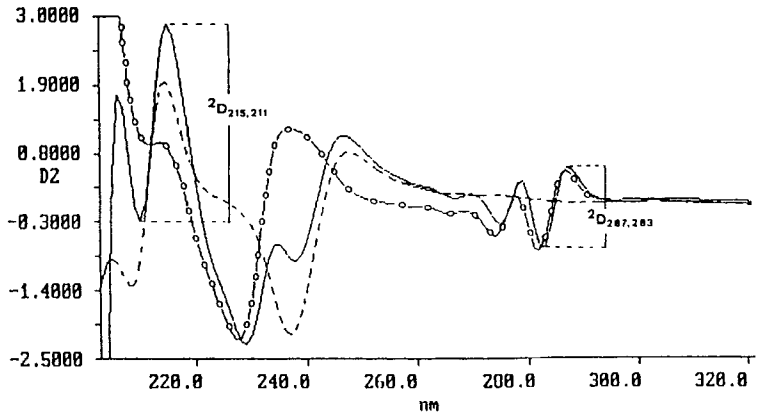

Fig.2 *Second derivative spectra of NF (- - -) 20.12 μg/mL, AT (-o-o-) 20.46 μg/mL and of their mixture 1:1 (——).*

Table I

Calibration graphs for atenolol and nifedipine determination

Method	Drug	Signal	Slope (± SD)	Intercept (± SD)	r
UV	AT	$^2D_{287,283}$	13.88 (0.30)	2.23 (0.47)	0.9997
	NF	$^2D_{215,211}$	6.02 (0.12)	1.65 (0.29)	0.9997
GLC	AT	$A_{AT}/A_{I.S.}$	394.63 (13.53	-4.32 (3.25)	0.9951
	NF	$A_{NF}/A_{I.S.}$	268.48 (12.80	3.82 (2.99)	0.9955

AT and NF concentrations are expressed as μg /mL.

Fig. 2 shows the second-order derivative spectra of AT, NF and of their mixture. The mixture spectrum presents the 287-283 nm peak-trough whose amplitude values is due only to the AT concentration, the contribution of NF being negligible at this wavelength. This signal amplitude and the drug concentration were correlated through the regression equation reported in Table I.

In the same spectrum the peak-trough 215-211 nm was found to be proportional to the NF concentration and not influenced by AT, since the absorbance contributions of this on the positive and negative peaks of the derivative signal present the same value. NF determination was performed by correlating the drug concentration with this signal through the regression equation reported in Table I.

A new gaschromatographic procedure was also developed to convalide the spectrophotometric results. The method is suitable as a routine analysis for the simultaneous determination of both substances in pharmaceuticals or biological samples.

At reported conditions, retention times (min) were: NF 7.7 ± 0.05; AT 5.9 ± 0.07; I.S. 2.9 ± 0.06 (Fig. 3).

The products concentrations were carried out through correlation with the area ratios value between analytes and internal standard. The respective regression equations are reported in Table I.

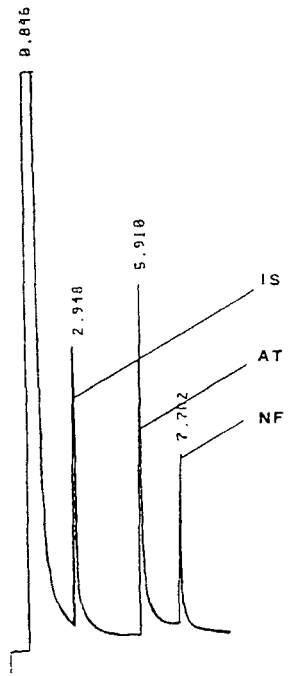

Fig.3 *Gaschromatogram of a standard solution, containing NF 0.73 mg/mL, AT 0.86 mg/mL and aminophylline as I.S. 1 mg/mL, in an injection volume of 1 μL.*

Table II

Determination of atenolol and nifedipine in laboratory mixtures and in pharmaceuticals

	Nominal			Found			
				UV method		GLC method	
Sample	AT	NF	AT/NF	AT (RSD%)	NF (RSD%)	AT (RSD%)	NF (RSD%)
Laboratory mixtures							
1	25.95	42.02	0.62	25.26 (0.65)	42.43 (0.15)	24.86 (1.56)	40.76 (0.56)
2	24.80	21.01	1.18	23.93 (0.35)	21.50 (0.98)	24.76 (0.77)	21.15 (0.62)
3	38.92	21.01	1.85	38.60 (0.13)	20.10 (0.52)	39.02 (0.78)	20.97 (1.37)
4	51.90	21.01	2.50	52.22 (0.56)	21.39 (0.65)	52.03 (0.47)	21.10 (0.85)
5	38.92	10.50	3.70	39.27 (1.50)	10.70 (1.10)	38.98 (0.82)	9.98 (1.04)
6	51.90	10.50	4.90	52.57 (1.22)	10.31 (0.78)	52.46 (2.13)	9.98 (1.46)
7	51.90	5.25	9.90	52.03 (0.77)	5.06 (0.87)	52.16 (1.38)	5.30 (0.48)
Pharmaceuticals							
Niften	50.00	20.00	2.5	49.92 (2.45)	20.83 (0.90)	48.64 (2.05)	21.06 (1.45)
Mixer	50.00	20.00	2.5	49.08 (1.75)	20.64 (2.03)	49.56 (1.20)	20.14 (2.34)

AT and NF concentrations are expressed as μg /mL.

VALIDATION

The satisfactory degree of linearity between the product concentrations and the measured values was confirmed by the correlation coefficients as being not less than 0.998.

To estimate if the equations of the UV method are free from errors due to the interference of the second component, a t test on the intercept was performed. t was carried out by the expression $t = a/s_a$, where a is the experimental intercept value and s_a its standard deviation. The calculated t values (2.24 for NF and 1.89 for AT) resulted less than the t critical value (t= 2.31, P= 0.05, d.f. 8) when the ratio AT/NF was between 0.5 and 6.

Recovery, expressed as percent of nominal amount, and precision (RSD%), carried out on binary mixtures in different percentages, resulted 99.12 (±1.87%) for NF and 98.23 (± 2.31%) for AT in the UV method; for gaschromatographic method the values were 97.56 (± 3.41%) for NF and 98.44 (± 2.89%) for AT.

The results of NF and AT determinations by both methods carried out on laboratory mixtures and commercial specialities are reported in Table II.

The accuracy of the UV method was estimated by performing a t test on laboratory mixtures containing a constant amount of AT and NF, in order to decide whether the difference between the nominal value and the experimental mean was significant. The calculated t values (1.87 for AT and 2.01 for NF) resulted less than the critical value (t= 2.57; d.f. 5) at the significance level of 0.05, so the "null hypothesis" is retained.

These very simple and rapid methods are suitable for routine quality control of pharmaceutical specialities.

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