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Fourth UV derivative spectrophotometry for the simultaneous assay of atenolol and chlorthalidone in pharmaceuticals

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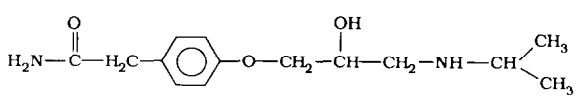
Summary

A rapid derivative spectrophotometric method for the simultaneous determination of atenolol and chlorthalidone, in antihypertensive specialities, is described. The analytical procedure is based on the linear relationship between the atenolol concentration and the minimum peak at 236 nm and that between the chlorthalidone concentration and the two signals arising from the minimum peak at 236 nm and a peak-trough at 229–218 nm. Satisfactory values for the precision and accuracy of the method have been estimated.

Introduction

Atenolol, 4-(2-hydroxy-3-isopropylaminopropoxy)phenyl acetamide (**I**), is an antihypertensive drug acting as a β-adrenoceptor antagonist.

Chlorthalidone, 2-chloro-5-(3-hydroxy-1-oxoisoindolin-3-yl)benzenesulphonamide (**II**), is a diuretic drug whose action is similar to those of other thiazides.



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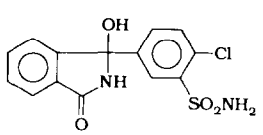

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The combined oral administration of the two drugs has been found to be more effective than either drug administered alone in the treatment of hypertension. Several commercial preparations contain the two drugs in association.

For atenolol assay, the British Pharmacopoeia (1988) reports a method of non-aqueous titration. In pharmaceutical forms the drug has been determined by NMR spectrometry (Iorio et al., 1987) and by a colorimetric method (Korany et al., 1985), while GLC, HPLC and spectrofluorometric techniques have been employed for determinations in human urine and blood serum (Malbica and Mouson, 1975; Scales and Copsey 1975; Weddle et al., 1978; Yee et al., 1979).

Chlorthalidone in both the British (1988) and Italian Pharmacopoeia (1985) is assayed by non-aqueous potentiometric titration, while a chromatographic procedure is reported in USP XX (1980).

In pharmaceutical forms, chlorthalidone has been determined by HPLC (O'Hare et al., 1979; Bauer et al., 1983) and UV spectrophotometry

(Peterkova et al., 1980; Vetuschi et al., 1985); in biological fluids, analyses have been performed according to GLC (Degen and Schweizer, 1977; Li et al., 1977; Fleuren and Van Rossum, 1978), HPLC (Guelen et al., 1980) and spectrophotometric (Tweeddale and Ogilvie, 1974) procedures.

Only a single method has been proposed, by Ficarra and co-workers (1985), which allows the simultaneous determination of two products in a pharmaceutical form by an HPLC procedure.

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

Experimental

Apparatus and conditions

Absorption and derivative spectra were recorded over the wavelength range 340–210 nm in 10 mm silica quartz cells using a Perkin-Elmer 320 UV-visible spectrophotometer. The scan speed was 1 nm s^{-1}, the response (time constant) 1 s, and the spectral bandwidth 1 nm. For the fourth derivative Δh was 8 nm.

Recording scales were: 10 nm cm^{-1} for the x-axis; as most suitable, for the y-axis. All amplitude measurements (expressed as mm) were referred to a scale of ± 1.

Reagents and chemicals

Ethanol (95% v/v) was spectroscopic reagent grade (C. Erba). Pure drugs, utilized as standards, were kindly supplied by Lisapharma S.p.A., Erba (Como), Italy.

Commercial preparations of pharmaceuticals

The following were examined: Diube (IBI, Italy; lot 75 M, Feb. 1988); Diube 50 (IBI, Italy; lot 03 M, Jan. 1988); Eupres (Schiapparelli Farmaceutici, Italy; lot 8599, Sept. 1988); Eupres mite (Schiapparelli Farmaceutici, Italy; lot 8720, May 1988); Igroseles (Farmitalia Carlo Erba, Italy; lot C8012, June 1988); Igroseles mite (Farmitalia Carlo Erba, Italy; lot C8003; Aug, 1988); Tenolone (Lusofarmaco, Italy; lot 02, June 1988); Target (Lisapharma, Italy); lot 464, Jan. 1988); Tenoret 50 (Stuart Pharmaceuticals, U.K.; 1987); Tenoretic (ICI Pharma, Italy; lot 817 F, June 1988); Tenoretic mite (ICI Pharma, Italy; lot 85 D, June 1988).

Standard solutions

Standard solutions were prepared in ethanol with atenolol concentrations in the range 5–60 μg ml^{-1} and with chlorthalidone concentrations varying between 5 and 30 μg ml^{-1}. The concentration ratio of **I/II** ranged from 0.4 to 10.

Thirty solutions were prepared in the above manner and were analyzed and used to obtain the reported regression equations.

Spectrophotometric analyses were performed against ethanol as a blank.

Sample solutions (a) Pharmaceuticals: Ten tablets were weighed and reduced to a fine powder. An amount corresponding to one tablet, accurately weighed, was transferred to a 100 ml volumetric flask, stirred with ethanol and made up to the above volume with the same solvent. 1 ml of the suspension was transferred to a 25 ml volumetric flask and diluted to volume with ethanol.

(b) Laboratory mixtures: Powder mixtures of the two products were prepared in order to obtain drug ratios (**I/II**) within the range 1–8; the following excipients were added in the usual amount of pharmaceutical formulations: talc, starch, magnesium stearate, titanium dioxide, magnesium carbonate. An amount of powder, accurately weighed, was diluted with ethanol to yield concentrations ranging from 5 to 60 μg ml^{-1} for atenolol and chlorthalidone at the corresponding concentration.

These solutions were utilized to establish the validity of the method.

Results and Discussion

Fig. 1 shows the absorption spectra of atenolol and chlorthalidone at similar concentrations in

ethanol. The spectra clearly display considerable overlap. Both compounds in fact show two maxima at 275 and 283 nm, with comparable values of absorptivity. A third maximum for atenolol occurs at 226 nm, whereas chlorthalidone in the same spectral zone exhibits a shoulder which is superimposed upon this peak with a higher absorptivity value. Despite these features, the absorption spectrum of the mixture is of no use for analytical purposes.

Similarly, the first-to-third derivative spectra show no improvement, since they are also very alike, and no signal is available that is directly correlated with the concentration of either of the two products.

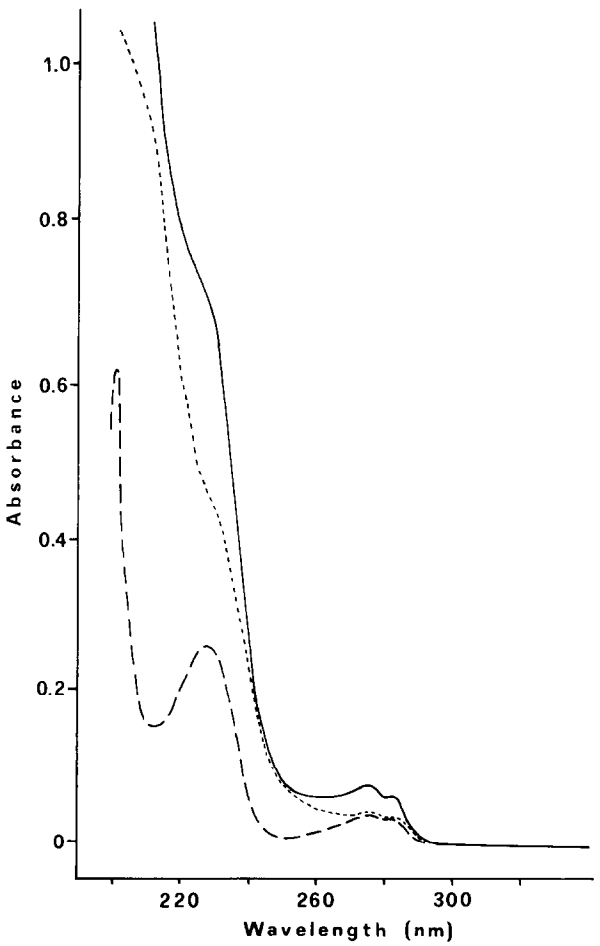

Fig. 1. Absorption spectra of atenolol (– – –) (10.09 μg ml^{-1}) and chlorthalidone (------) (10.12 μg ml^{-1}).

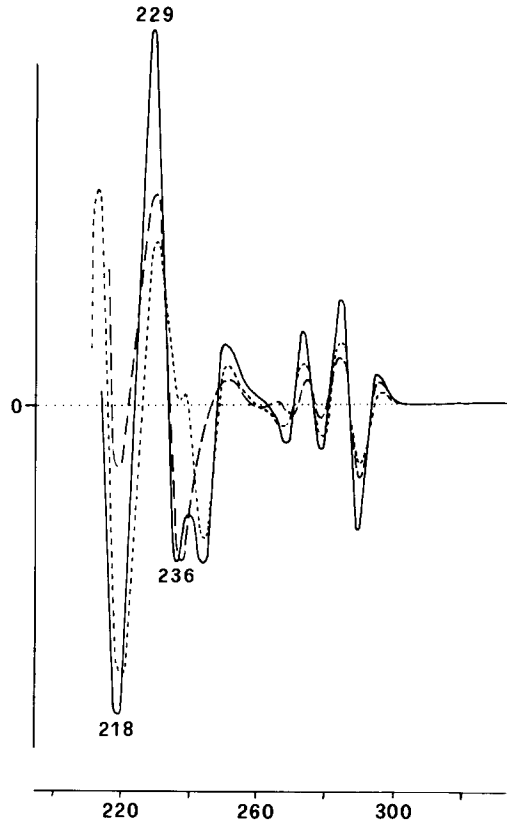

Fig. 2. Fourth derivative spectra of atenolol (– – –), chlorthalidone (------) and their 1 : 1 mixture (—).

In the fourth derivative spectrum (Fig. 2), on the other hand, a peak-trough was observed, whose minimum at 236 nm is proportional to the atenolol concentration within the range for the standard solutions previously reported.

This feature is not evident for chlorthalidone, its corresponding value being negligible at this wavelength.

With regard to chlorthalidone, since the spectrum shows no signal allowing its direct evaluation, the drug concentration is determined by using, at the same time, the previously reported signal at 236 nm, and the peak-trough between 229 and 218 nm, principally influenced by chlorthalidone. The chlorthalidone concentration has been correlated vs these two values through a multiple linear regression procedure.

Atenolol determination

The atenolol assay is performed by using the amplitude value for the minimum peak at 236 nm, with respect to the baseline, through the following regression equation:

$$A = 0.1060\left({}^4D_{236\ \min}\right) + 0.6575 \quad r = 0.99995$$

where A (atenolol) is expressed as μg ml^{-1} and ${}^4D_{236\ \min}$ as mm on the scale ± 1.

Chlorthalidone determination

By using the values of the peak-trough at 229-218 nm and the peak minimum at 236 nm, the following regression equation gives the concentration of chlorthalidone:

$$C = 0.03903\left({}^4D_{229,218}\right) - 0.06604\left({}^4D_{236\ \min}\right) + 1.5801 \quad r = 0.99988$$

where C (chlorthalidone) is expressed as μg ml^{-1} and peak-trough amplitudes ${}^4D_{229,218}$ and ${}^4D_{236\ \min}$ as mm on the scale ± 1.

The proposed method has been applied in determinations on laboratory mixtures of the pure drugs and on several commercial specialities.

Validation

The linearity between the measured values and the drug concentrations was verified by means of constructing a six-point calibration curve at concentrations in the range described for the laboratory mixtures. For each concentration, measurements were repeated five times, replenishing the cells each time with fresh solution.

The satisfactory degree of linearity was confirmed by the correlation coefficients being not less than 0.9998.

Table 1 lists the results of atenolol and chlorthalidone determinations (means of five values) and the relative precision values, expressed as RSD%, carried out on laboratory mixtures and commercial tablets.

The data on the recovery, expressed as percent of nominal amount, estimated for laboratory mixtures, were 99.29 $\pm$ 1.24% for atenolol and 99.12 $\pm$

TABLE 1

Determination results and precision values in analyses of laboratory mixtures and commercial preparations

Sample	Nominal		Found			
	I	II	I	RSD%	II	RSD%
Laboratory mixtures						
1	20.18	20.24	20.23	0.31	21.15	0.43
2	20.08	10.10	20.18	0.23	10.15	0.36
3	40.36	10.12	40.90	0.43	10.71	0.51
4	40.08	5.02	39.63	0.45	4.92	0.18
Pharmaceutical preparations						
Diube	100	25	96.94	1.14	25.64	2.74
Diube 50	50	12.5	51.70	2.03	13.87	1.61
Eupres	100	25	101.90	0.98	24.39	3.56
Eupres mite	50	25	51.46	1.03	25.74	4.12
Igroseles	100	25	100.36	1.60	24.33	2.12
Igroseles mite	50	12.5	49.98	1.74	11.99	3.88
Target	100	25	99.98	2.97	25.02	3.33
Tenolone	100	25	96.90	0.79	25.09	2.72
Tenoret 50	50	12.5	49.63	0.91	12.43	2.73
Tenoretic	100	25	97.40	2.20	23.98	2.99
Tenoretic mite	50	12.5	48.96	0.83	12.12	4.05

All values (μg/ml) are means of five determinations.

2.90% for chlorthalidone (means of 10 determinations ± SD).

The high degree of accuracy and precision calculated is in agreement with the satisfactory results obtained by using the proposed method. This very simple and rapid procedure, moreover, is suitable for routine analyses in quality control.

Acknowledgement

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