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Determination of irbesartan in the presence of hydrochlorothiazide by derivative spectrophotometry

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Abstract

A first-derivative spectrophotometric method for the determination of irbesartan (IRB) alone and in the presence of hydrochlorothiazide (HCT) is described. Measurements are made at the zero-crossing wavelength at 263.0 nm for IRB. The calibration graph was linear over the range 1.0–12.0 mg l^{-1} of IRB, detection limit was 0.15 mg l^{-1}. HCT, in the presence of IRB was determined by direct spectrophotometric method at 317 nm. The calibration graph was linear over the range 2.0–50.0 mg l^{-1} of HCT, detection limit was 0.25 mg l^{-1}. The proposed methods were successfully applied to the determinations of IRB and HCT in combined tablets.

Keywords: Irbesartan; Hydrochlorothiazide; Derivative spectrophotometry; Pharmaceuticals

1. Introduction

Irbesartan, 2-butyl-3-[[2′-(1H-tetrazole-5-yl)(1,1′-biphenyl)-4-yl]methyl]-1,3-diazaspiro[4,4]non-1-en-4-one, (IRB) is a potent, long-acting angiotensin II (AII) receptor antagonist, with high selectivity for the AT_1 subtype [1]. Its chemical structure is shown in Fig. 1. IRB and other AII receptor antagonists are potentially safe and more tolerable than earlier classes of drugs used for the treatment of hypertension, diabetic nephropathy and heart failure. A clinical study in hypertensive subjects has demonstrated that IRB effectively lowers blood pressure with a once daily dosage and its biotransformation mechanism in man has been determined [2]. IRB was marketed in 1998 and is administered alone or reinforced with the antihypertensive diuretic, hydrochlorothiazide (HCT).

The pharmacodynamic, pharmacokinetic properties and therapeutic use of IRB have recently been reviewed [3]. However, to our knowledge, no method has been described for its determination, despite the obvious convenience of having an accurate, precise and reliable method for determining IRB alone or in the presence of HCT.

The direct UV–VIS spectrophotometric method presents a severe problem in that the

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spectral bands of IRB and HCT overlap, making it unsuitable for the determination of IRB in the presence of HCT.

Derivative UV–VIS spectrophotometry involves calculating and plotting one of the mathematical derivatives of a spectral curve, which offers an alternative approach to drug analysis. Although the derivative transformation does not increase the information content of a given spectrum, this method shows good sensitivity and specificity and permits discrimination in the face of the broad band interference arising from turbidity or non-specific matrix absorption. Several papers on the theoretical aspects of derivative spectrophotometry have been published [4–7] and the recognized resolution enhancement of derivative UV–VIS spectrophotometry has been used advantageously in the determination of drugs in biological fluids [8–13] and binary mixtures of drugs [14–20].

In this paper, the ease with which the derivative methods circumvent the problem of overlapping spectral bands is demonstrated, allowing the determination of IRB alone and in the presence of HCT without prior separation, and providing accurate, precise, rapid and reproducible results.

The HCT, in the presence of IRB, can be determined by UV–VIS spectrophotometric method at 317 nm, a wavelength at which IRB does not absorb. This method is applied for the determination of HCT in the presence of IRB in this paper.

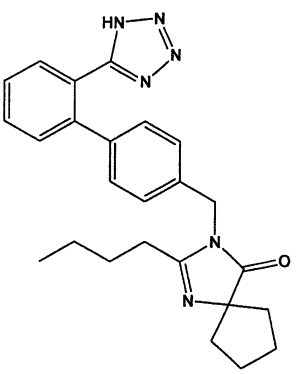

Fig. 1. Chemical structure of IRB.

2. Experimental

2.1. Apparatus

A Shimadzu (Kyoto, Japan) UV 240 double-beam spectrophotometer with an optional program unit (model OPI-2) and 1 cm quartz cell was used. The most suitable settings were: slit width, 2 nm (the response time was automatically adjusted according to the slit width); scan speed, 45 nm min^{-1}; derivative wavelength difference, 4 nm. The recorder scale expansion was optimized to facilitate readings on the recorder trace.

2.2. Standard solutions

All chemicals and solvents were of analytical-reagent grade and the solutions were prepared with doubly distilled water.

IRB stock standard solution (100.0 mg l^{-1}) was prepared by dissolving 10.0 mg of IRB (kindly provided by Lab. Bristol-Myers Squibb, Madrid, Spain) in 100 ml of absolute ethanol. HCT stock standard solution (100.0 mg l^{-1}) was prepared by dissolving 10.0 mg of HCT (Sigma, St Louis, MO, USA) in 100 ml of absolute ethanol. Working standard solutions were prepared by suitable dilution of the corresponding stock standard solutions with absolute ethanol.

2.3. Dosage forms

The dosage forms were: (1) Karvea tablets (Bristol-Myers Squibb Lab.) 75 mg of IRB, Karvezide tablets (Bristol-Myers Squibb Lab.) 150 mg of IRB and 12.5 mg of HCT and Coaprovel tablets (Sanofi Winthrop Lab., Spain) 300 mg of IRB and 12.5 mg of HCT. The excipients were cellulose, lactose, starch, magnesium stearate, colloidal silica, sodium croscarmelose, poloxamer 88, red and yellow iron oxides to make up the total weight of the tablet in every one of the pharmaceuticals.

2.4. Procedures

2.4.1. Calibration procedures

For IRB determination, different volumes (100

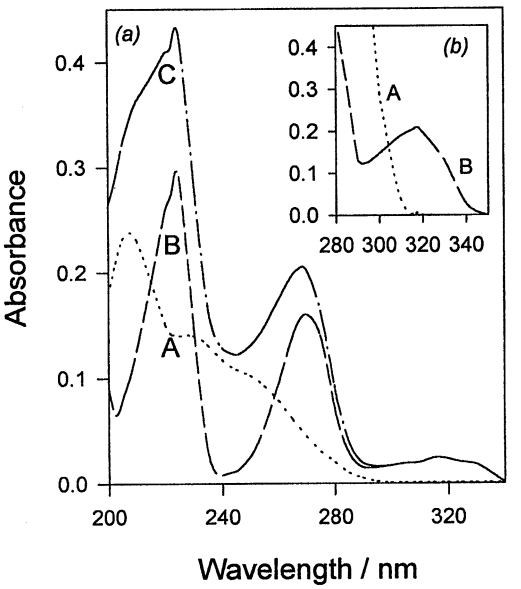

Fig. 2. (a) Absorption (zero-order) spectra in ethanolic medium of: (A) 3.0 mg l^{-1} of IRB; (B) 2.5 mg l^{-1} of HCT; (C) 3.0 mg l^{-1} of IRB and 2.5-mg l^{-1} of HCT. (b) Absorption (zero-order) spectra in ethanolic medium of: (A) 400.0 mg l^{-1} of IRB; (B) 20.0 mg l^{-1} of HCT.

μl–2.00-ml) of standard stock solution of IRB and 200 μl of standard stock solution of HCT were simultaneously placed in a 10 ml calibrated flask and diluted with ethanol to give a final concentration of between 1.0–20.0 mg l^{-1} of IRB and 2.0 mg l^{-1} of HCT. The first order derivative spectrum of these solutions were recorded over the wavelength range of 200–300 nm. The amplitude (mm) was measured at 263 nm for IRB.

For HCT determination, different volumes (200 μl–6.00 ml) of standard stock solution of HCT and 4.00 ml of standard stock solution of IRB were simultaneously placed in a 10 ml calibrated flask and diluted with ethanol to give a final concentration of between 2.0 and 60.0 mg l^{-1} of HCT and 40.0 mg l^{-1} of IRB. The absorbance of these solutions at 317 nm in the zero order spectrum was measured.

2.4.2. Procedure for determination of irbesartan and hydrochlorothiazide in pharmaceuticals

The average tablet weight was calculated from the contents of ten tablets that had been finely powdered and weighed. Aliquots of this powder, equivalent to 10 mg of IRB, or to 10 mg of HCT, were accurately weighed. The different samples were shaken with 25 ml of ethanol and the mixtures were then introduced into an ultrasonic bath for 10 min, filtered through a Millipore filter. The filtrate was diluted with ethanol in a 100 ml calibrated flask and the described calibration procedures for the IRB or HCT determination were applied.

3. Results and discussion

3.1. Spectrophotometric measurements

In Fig. 2(a) the zero-order spectra of 3.0 mg l^{-1} of IRB (A), 2.5 mg l^{-1} of HCT (B) and their mixture (C), in the wavelength range 200–325 nm, are shown. IRB exhibits a defined absorbance maximum at 207 nm and two wide absorption bands between 220–230 and 250 nm. The HCT exhibits three absorbance maxima at 225, 270 and 317 nm. As can be seen, that of the HCT spectrum substantially overlaps the absorption spectrum of IRB, which prevents the determination of the IRB in the presence of HCT by direct UV–VIS absorbance measurements.

Derivative spectrophotometry is a suitable technique for overcoming this problem, with the zero-crossing method being the most common procedure for the preparation of analytical calibration graphs. In practice, the measurements selected are those which exhibit the best linear response, give a zero or near zero intercept on the ordinate of the calibration graphs and which are least affected by the concentration of any other component.

Fig. 3 shows the first derivative UV–VIS spectra of the same solutions of the Fig. 2(a). Derivatization of the zero-order spectra leads to an improvement of the spectral details, while the resulting first derivative spectra present spectral features, which can be used for the determination of IRB in the presence of HCT. Due to the overlapping spectra, the zero-crossing method is

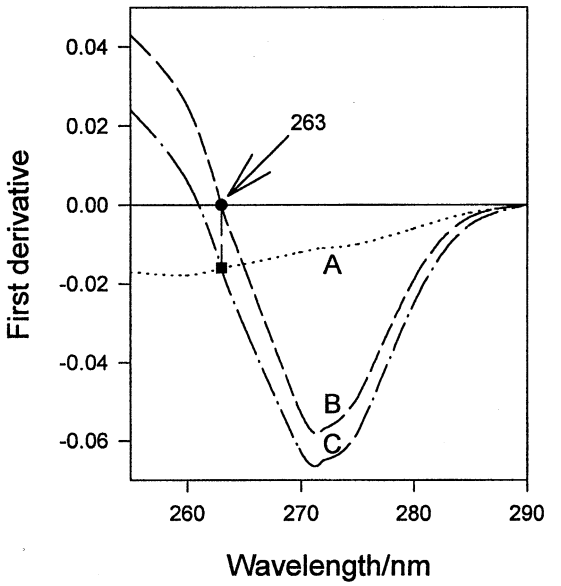

Fig. 3. First-derivative spectra in ethanolic medium ($\Delta\lambda = 4$ nm) of: (A) 3.0 mg l^{-1} of IRB; (B) 2.5 mg l^{-1} of HCT; (C) 3.0 mg l^{-1} of IRB and 2.5 mg l^{-1} of HCT.

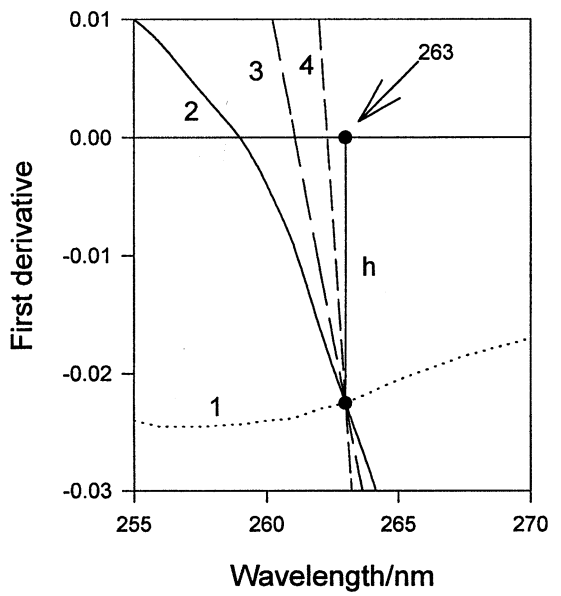

Fig. 4. First-derivative spectra in ethanolic medium ($\Delta\lambda = 4$ nm) of samples containing 4.0 mg l^{-1} of IRB and different concentrations of HCT (1) 0.0; (2) 2.0; (3) 4.0; (4) 8.0-mg l^{-1}.

clearly the most appropriate approach for resolving mixtures of these compounds and, as such, was used in this work with satisfactory results. Preliminary experiments showed that the signals of first derivative at 263.0 nm (working zero-crossing wavelength of HCT) are proportional to the IRB present.

3.2. Selection of optimum instrumental conditions

The main instrumental parameters that affect the shape of the derivative spectra are the wavelength scanning speed, the wavelength increment over which the derivative is obtained ($\Delta\lambda$) and the smoothing, all of which need to be optimized to give a well resolved large peak. Generally, the noise level decreases as $\Delta\lambda$ increases, which leads to less pronounced fluctuations in the derivative spectrum. Since spectral resolution is very poor at excessively high $\Delta\lambda$ values, the optimum value of $\Delta\lambda$ should be determined by taking into account the noise level, the resolution of the spectrum and the sample concentration. Several values of $\Delta\lambda$ were tested and 4.0 nm was selected as the optimum for a satisfactory signal-to-noise ratio.

After establishing the optimum experimental conditions, the first derivative spectra of a serie of samples containing different concentrations (0.0–8.0 mg l^{-1}) of HCT, and a constant concentration (4.0 mg l^{-1}) of IRB were recorded in order to investigate the effect of HCT on the determination of the IRB. Fig. 4 shows that the height h was not affected by the presence of HCT over the range of concentrations studied in the first-derivative mode

3.3. Features of the method for the determination of irbesartan

Some binary mixtures of IRB and HCT in ethanolic solutions were prepared from the working standard solutions by keeping the HCT concentration constant at 2.0 mg l^{-1} while the IRB concentration was varied from 0.0 to 20.0 mg l^{-1}. The first order derivative spectra of these samples were recorded over the wavelength range 200–300 nm against a blank of ethanol. The calibration graph was constructed by plotting the analytical

signals ${}^1D_{IRB}$ at 263.0 nm versus the IRB concentration (mg l^{-1}). The ordinate values, ${}^1D$, of the equations were calculated from the amplitude measurements (mm) and standardized as follows: ${}^1D$ = recorder divisions (h mm) × scale expansion/150 mm full-scale [21].

The calibration graph was linear between 1.0 and 12.0 mg l^{-1} of IRB. The regression equation found was ${}^1D_{263} = -9.2 \times 10^{-5}$ ($\pm 1.0 \times 10^{-4}$) + 5.4×10^{-3} ($\pm 1.9 \times 10^{-5}$) [IRB] where the IRB concentration is expressed in mg l^{-1} with a correlation coefficient of 0.9999. The detection and quantification limits achieved, as defined by IUPAC [22], were 0.15 and 0.49 mg l^{-1} of IRB, respectively.

The within-run precision of the method was evaluated by analysing five replicates of two series of samples containing simultaneously 10.0 or 2.0 mg l^{-1} of IRB and 2.0 mg l^{-1} of HCT. The variation coefficients obtained were ± 0.7 or $\pm$ 1.0%, respectively. The between-run precision of the method was evaluated with two series of samples containing the concentrations of the above drugs being analyzed during 5 consecutive days. The variation coefficients obtained were ± 0.9 or ± 1.3%, respectively.

The influence of frequently encountered excipients in pharmaceutical dosage forms of IRB on the proposed method was studied by adding different amounts of possible interferents to samples containing 10.0 mg l^{-1} of IRB or 10.0 mg l^{-1} of IRB and 2.0 mg l^{-1} of HCT. No interference was observed from the presence of cellulose, lactose, starch, magnesium stearate, and colloidal silica, in the amounts commonly contained in the pharmaceuticals assayed.

3.4. Determination of hydrochlorothiazide in the presence of irbesartan

Fig. 2(b) shows the zero-order spectra of 400.0 mg l^{-1} of IRB (A) and 20.0 mg l^{-1} of HCT (B), in the wavelength range 280–350 nm. As can be seen at 317 nm (maximum absorption of HCT), the IRB does not absorb even at the high ratio assayed (20/1). The measurements of HCT absorbance at 317 nm were used for determining HCT in the presence of IRB. Binary mixtures of HCT and IRB in ethanolic solutions were prepared from the working standard solutions by keeping the IRB concentration constant at 40.0 mg l^{-1} while the HCT concentration was varied from 0.0 to 60.0 mg l^{-1}. The calibration graph was linear between 2.0 and 50.0 mg l^{-1} of HCT. The regression equation found was $A = -2.4 \times 10^{-3}$ ($\pm 0.8 \times 10^{-3}$) + 1.0×10^{-2}($\pm 3.3 \times 10^{-5}$) [HCT] where the HCT concentration is expressed in mg l^{-1} with a correlation coefficient of 0.9999. The detection and quantification limits, was 0.26 and 0.88 mg l^{-1} of HCT, respectively.

The within-run precision of the method was evaluated by analyzing five replicates of two series of samples containing simultaneously 5.0 or 20.0 mg l^{-1} of HCT and 40.0 mg l^{-1} of IRB. The variation coefficients obtained were ± 1.3 or ± 1.1%, respectively. The between-run precision of the method was evaluated with two series of samples containing the concentrations of the above drugs being analyzed during 5 consecutive days. The variation coefficients obtained were ± 1.7 or ± 1.8%, respectively.

3.5. Applications

The proposed methods were successfully applied to the analysis of different pharmaceutical dosage forms containing IRB alone or IRB and HCT. The results are summarized in Table 1. When different pharmaceuticals of IRB alone or in the presence of HCT were analysed by the proposed methods, interference from the sample matrix posed no problem. For all the formulations examined, the methods gave results, which were in good agreement with the declared content.

The validity of the methods was confirmed by applying the standard additions technique to the different pharmaceuticals containing IRB alone and jointly with HCT. The results for IRB and HCT obtained are shown in Tables 2 and 3, respectively. As can be seen, recoveries of 97.3–101.4 and 99.2–103.6% were obtained for IRB and HCT, respectively.

Table 1
Determination of IRB and HCT in pharmaceuticals

Sample	Irbesartan[a]		Hydrochlorothiazida[a]	
	Labelled	Found[b]	Labelled	Found [b]
Karvea	75	73.65 (±0.69)	–	–
Karvezide	150	150.19 (±1.12)	12.5	12.42 (±0.26)
Coaprovel	300	288.00(±2.71)	12.5	12.57 (±0.17)

[a] Content (mg per tablet).
[b] Mean of six determinations (±S.D.).

4. Conclusions

The proposed first-derivative spectrophotometric method is suitable for the determination of IRB alone and in the presence of hydrochlrothiazide. The method is simple, accurate and precise and is useful for the quality control and routine analysis.

It should be noted that no analytical method for the determination of IRB was found in the bibliography.

Table 2
Recoveries of IRB in pharmaceuticals

Sample	Irbesartan (mg l^{-1})		
	Taken	Added	% Recovery[a]
Karvea	1.96	2.00	99.7 (±0.6)
Karvea	3.92	3.00	100.0 (±0.5)
Karvezide	5.01	4.00	101.2 (±0.9)
Karvezide	5.01	5.00	99.6 (±0.7)
Coaprovel	0.96	5.00	101.4(±0.8)
Coaprovel	2.88	4.00	97.3(±0.6)

[a] Mean of six determinations (±S.D.)

Table 3
Recoveries of HCT in pharmaceuticals

Sample	Hydrochlorothiazide (mg l^{-1})		
	Taken	Added	% Recovery [a]
Karvezide	17.36	10.00	99.2 (±1.7)
Karvezide	8.68	20.00	100.9 (±1.6)
Coaprovel	8.34	10.00	103.6 (±1.0)
Coaprovel	16.68	20.00	101.7 (±0.7)

[a] Mean of six determinations (±S.D.).

Hydrochlrothiazide in the presence of IRB can be determined in pharmaceuticals by applying direct spectrophotometry.

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