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Three new spectrophotometric methods applied to the simultaneous determination of hydrochlorothiazide and irbesartan

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Received August 16, 2002, accepted February 23, 2003

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Pharmazie 58: 543–548 (2003)

This work involves the simultaneous determination of hydrochlorothiazide and irbesartan in a binary mixture without previous separation by three new analytical methods. The first method, based on compensation technique, is presented for the derivative spectrophotometric determination of binary mixtures with overlapping spectra. By using ratios of the derivative maxima or the derivative minimum, the exact contribution of either component in the binary mixture can be measured and the amounts quantified. The second method uses of the first derivative of the ratio spectra. The ratio spectra were obtained by dividing the absorption spectra of the binary mixture by that of one of the components. The amplitudes in the first derivative of the ratio spectra at 231, 266, 279, 238 and 248 nm were selected to determine hydrochlorothiazide and irbesartan in binary mixtures. The concentration of the other components are then determined from their respective calibration graphs treated similarly. With the third method, the absorbance ratio method, the determination of hydrochlorothiazide and irbesartan was performed using the absorbances read at 272 nm, 241 nm and 263 nm in the zero-order spectra of their mixture. The absorbance ratio was also developed as a comparison method. The three methods are simple, accurate, rapid and require no preliminary separation steps and can, therefore, be used for routine analysis of both drugs in quality control laboratories.

1. Introduction

Irbesartan, (2-butyl-3-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]1,3-diazaspiro[4,4]-non-1-en-4-one), is the first member of a new class of a non-peptide angiotensin II receptor antagonists. The first approved indication for irbesartan is hypertension. Recently, a new combination dosage form of irbesartan and hydrochlorothiazide, a diuretic, has been indicated in the treatment and management of edema and hypertension.

Only one HPLC method has been reported for the determination of irbesartan in human plasma and urine [1]. A number of methods have been reported for the determination of hydrochlorothiazide, individually or in combination, in pharmaceutial formulations or in biological fluids, such as by voltammetry [2], capillary zone electrophoresis [3–5], spectrophotometry [6–14] and HPLC [15–21].

Molecular absorption spectroscopy has been extensively used for the quantitative determination of drugs in pharmaceutical preparations, as well as for the analysis of synthetic mixtures. The use of this technique for pharmaceutical analyses has the inherent disadvantage that most active drugs absorb in the UV region and exhibit strongly overlapped spectra that impede their simultaneous determination. This problem can be compensated by various methods including ratio derivative spectra and compensation technique. Compensation technique [22] is a useful technique for UV and IR spectrophotometric analysis of mixtures of compounds. It is a non-mathematical method for the detection and elimination of unwanted absorption during spectrophotometric analysis. In binary mixture analysis, the compensation method involves a comparison of several difference spectra (mixture-reference) using different concentrations of a reference solution in the reference cell. Hence, if A_m and A_r refer to the absorbances of the relevant cells against air at same wavelength λ, then $\Delta A_\lambda = A_{m\lambda} - A_{r\lambda}$, where $A_m = A_a + A_b$ at a given wavelength λ, a and b refer to components a and b, respectively, and A_r refers to A_a or A_b. If C_r for compound a is introduced into the reference cell, the absorption characteristics of the mixture gradually approach that of compound b as c_a increases and finally coincides with the absorption curve of compound b at the end-point, for which $c_r = c_a$, and by analogy c_b can be found by repeating the same steps using c_r for compound b in the reference cell. The accuracy of the method depends on the evaluation of the balance point.

Salinas et al. [23, 24] developed a new spectrophotometric method based on the use of the first derivative of the ratio spectra for resolving binary mixtures. This method permits the use of the wavelength where maximum change occurs i.e., at a maximum or a minimum as the signal of measurement. Moreover, the presence of many maxima and minima is a further advantage because these wavelengths provide additional selectivity for the determination of active compounds in the presence of other compounds and excipients that may interfere in the assay.

The hydrochlorothiazide-irbesartan mixture is not yet official in any pharmacopoeia. To my knowledge, neither a compensation technique, a ratio derivative spectrophotometric method nor an absorbance ratio method have been described for the simultaneous determination of both drugs in dosage forms. Therefore, there is a need for a new analytical methods that allow simultaneous determination.

The aim of this work was to investigate the utility of compensation technique and ratio derivative spectrophotometry in the assay of hydrochlorothiazide and irbesartan in combination in pharmaceutical preparations without the necessity of sample pre-treatment. The results obtained by the proposed methods were compared with those obtained by the absorbance ratio method.

2. Investigations, results and discussion

2.1. Compensation technique

The stability of the working solutions of hydrochlorothiazide and irbesartan was studied by recording the time-dependent absorption spectra; no changes in the spectra were observed for at least five days when the solutions are stored at room temperature in the dark. The simultaneous determination of hydrochlorothiazide and irbesartan by direct UV absorption measurements is not possible due to spectral overlap (Fig. 1a). Fig. 1b shows the second derivative spectra (^{2}D) of irbesartan and hydrochlorothiazide. The second derivative spectra were recorded for each reference solution of the analyte components and the ratios of the ^{2}D maximum and ^{2}D minimum or ^{2}D maxima and ^{2}D minimum were calculated. The influence of $\Delta\lambda$ on the second derivative spectra of hydrochlorothiazide and irbesartan was tested to obtain the optimum wavelength interval; $\Delta\lambda$: 4 nm was considered as suitable. Table 1 shows the mean values of the ratios calculated for ten separate

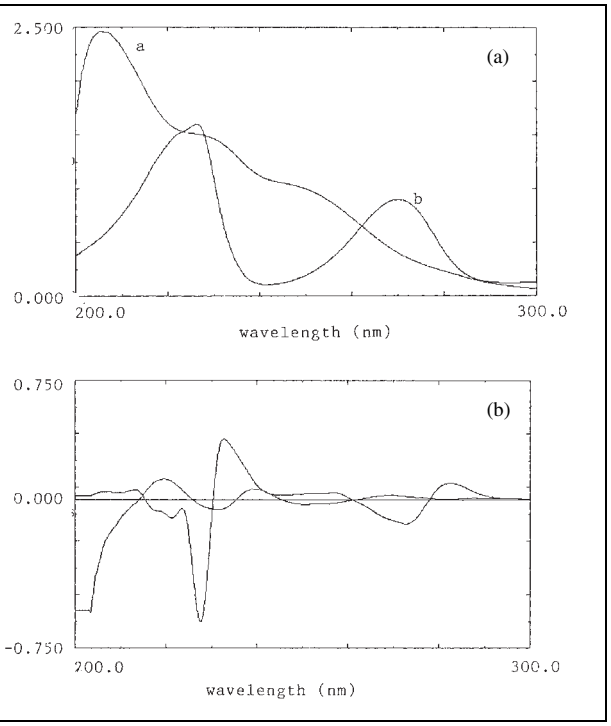

Fig. 1: (a): Zero-order spectra of a) 24.0 $\mu g \cdot ml^{-1}$ hydrochlorothiazide; and b) 55.0 $\mu g \cdot ml^{-1}$ irbesartan in methanol. (b): Second derivative spectra of a) 24.0 $\mu g \cdot ml^{-1}$ hydrochlorothiazide; and b) 55.0 $\mu g \cdot ml^{-1}$ irbesartan in methanol ($\Delta\lambda$: 4).

Table 1: Simultaneous determination of irbesartan and hydrochlorothiazide in binary mixture by the compensation method

Preparation	Linearity range ($\mu g \cdot ml^{-1}$)	Ratio	Mean*	RSD (%)
Irbesartan	10.0–50.0	^{2}D (231)/^{2}D(240)	0.998	1.129
Hydrochlorothiazide	4.0–24.0	^{2}D (228)/^{2}D(233)	0.785	0.655

* Mean of ten separate determinations

determinations with standard solutions. The ratios are constant, characteristic of the pure substance, independent of concentration and not affected by other absorbing components. For the determination of irbesartan concentrations in irbesartan-hydrochlorothiazide mixtures, the sample cell was filled with the mixture solution and the reference cell was filled, in succession, with a series of reference irbesartan solutions with different concentrations. The ratios of the mixture calculated from ratios are constant, characteristic of the pure substance, independent of concentration and not affected by other absorbing components. The ratios of the mixture calculated from the recorded ^{2}D spectra were compared with those of hydrochlorothiazide. At the balance point, the ratio of the mixture corresponds to that of hydrochlorothiazide, where the concentration of irbesartan in the mixtures in the sample cell is equal to that of the reference solution. For determining the other component, the same steps with solutions of pure compound hydrochlorothiazide in the reference cell were followed to determine its concentration in the mixture at the balance point. Conformity with Beer's law was evident in the concentration range from 10.0 to 50.0 $\mu g \cdot ml^{-1}$ of irbesartan and from 4.0 to 24.0 $\mu g \cdot ml^{-1}$ of hydrochlorothiazide. In this method, the synthetic mixtures were prepared by adding known amounts of irbesartan-hydrochlorothiazide. The selectivity of the method for the estimation of the drugs in the presence of various tablet excipients such as starch,

Table 2: Recovery experiments obtained for different binary mixtures of irbesartan (IRB) and hydrochlorothiazide (HYD) by the compensation technique

Sample	Recovery (mean ± sd) (%)[a]					
	Irbesartan			Hydrochlorothiazide		
	Amount added ($\mu g \cdot ml^{-1}$)	Amount found ($\mu g \cdot ml^{-1}$)	Recovery (%)	Amount added ($\mu g \cdot ml^{-1}$)	Amount found ($\mu g \cdot ml^{-1}$)	Recovery (%)
	100.0			12.5	12.3	98.4
	125.0			12.5	12.5	100.0
	150.0			12.5	12.4	99.2
	200.0			12.5	12.4	99.2
	225.0			12.5	12.7	101.6
	150.0	149.8	99.9	7.5		
	150.0	150.7	100.5	10.0		
	150.0	150.0	100.0	15.0		
	150.0	148.9	99.3	17.5		
	150.0	149.2	99.5	20.0		

	$\overline{X}$ (%)	R.S.D (%)
HYD	99.7	1.08
IRB	99.8	0.41

[a] Mean and standard deviation for five determinations; percentage recovery from the label claim amount

Table 3: Comparative studies for commercial preparations

	Mean (mg) ± SD[a]					
	Compensation technique		Ratio spectra derivative spectrophotometry		Absorbance ratio method	
	Irbesartan	Hydrochlorothiazide	Irbesartan	Hydrochlorothiazide	Irbesartan	Hydrochlorothiazide
Batch 1	150.2 ± 0.9 t: 0.94[b]	12.1 ± 0.9 t: 0.89	149.9 ± 0.5 t: 1.07	12.5 ± 0.4 t: 1.68	150.9 ± 1.4	12.5 ± 1.1
Batch 2	149.8 ± 1.8 t: 1.12	12.3 ± 0.5 t: 1.78	150.2 ± 1.0 t: 1.28	12.4 ± 1.3 t: 1.21	50.2 ± 0.6	12.5 ± 1.9
Batch 3	151.2 ± 1.7 t: 1.14	12.5 ± 0.6 t: 1.63	148.8 ± 1.4 t: 1.64	12.5 ± 1.4 t: 1.44	149.8 ± 0.9	12.8 ± 0.4

[a] Each value is the mean of ten experiments; SD = Standard deviation
[b] Values in parentheses are the theoretical values at p = 0.95. Theoretical values at % 95 confidence limits t = 2.26

lactose, talc and magnesium strearate was investigated. A placebo comprising starch 10%, lactose 40%, talc 2% and magnesium strearate 1% was prepared and mixed in a 1 : 1 ratio with the drug. Recoveries and relative standard deviations of the method were found as 99.8 and 0.41% for irbesartan and 99.7 and 1.08% for hydrochlorothiazide in a binary mixture (Table 2). The results with the synthetic mixture encouraged me to use the method to assay for irbesartan and hydrochlorothiazide in commercial tablet dosage forms. The recovery of irbesartan and hydrochlorothiazide in three batches of commercial formulations was measured. The results presented in Table 3 are in good agreement with the labelled content. All data represent the average of five determinations and the small relative standard deviations indicate good precision.

2.2. Ratio spectra derivative spectrophotometry

A new spectrophotometric method is described for the quantitative analysis of binary or ternary mixtures with overlapping spectra. The method is based on the use of the first derivative of the ratio spectra. The ratio spectra were obtained by dividing the absorption spectrum of the mixture by that of one of the components. The absorption spectra of the two components are strongly overlapped (Fig. 1a). This spectral overlapping was sufficiently enough to demonstrate the resolving power of the proposed method. Fig. 2 shows the ratio spectra of different irbesartan standards (spectra divided by the spectrum of a 16.0 $\mu g \cdot ml^{-1}$ hydrochlorothiazide solution) and their first derivatives.

The ratio first derivative amplitudes at 238 ($^1DD_{238}$) and 248 ($^1DD_{248}$) nm, corresponding to a maximum and minimum wavelengths, are proportional to the irbesartan concentration. For determining hydrochlorothiazide, the stored spectra of the mixtures are divided by a standard spectrum of irbesartan of 20.0 $\mu g \cdot ml^{-1}$. In the same way as describe above, the content of hydrochlorothiazide was determined by selecting the first derivative of the ratio spectrum in the range 200.0–300.0 nm and measuring the signals at 230 nm ($^1DD_{230}$), 266 nm ($^1DD_{266}$), and 279 ($^1DD_{279}$) nm, Fig. 3. The optimal wavelengths for the

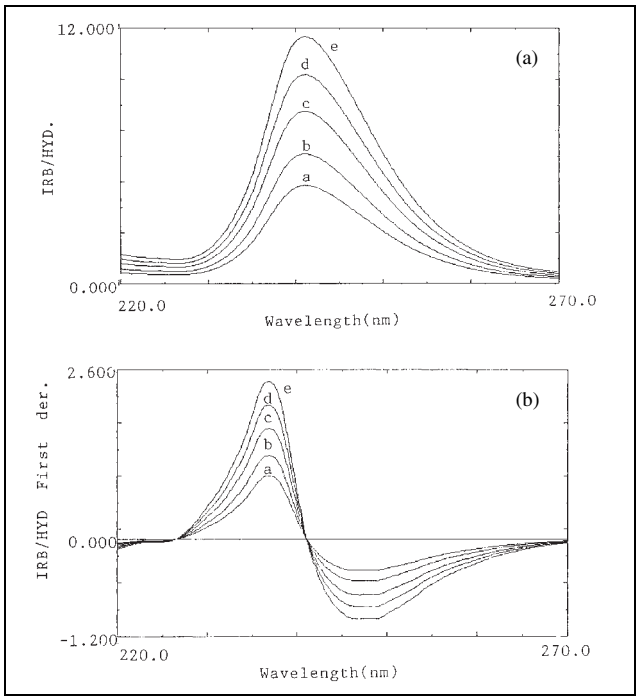

Fig. 2: Ratio spectra (a) and first derivative of the ratio spectra (b) of irbesartan of a) 10.0 $\mu g \cdot ml^{-1}$, b) 20.0 $\mu g \cdot ml^{-1}$, c) 30.0 $\mu g \cdot ml^{-1}$, d) 40.0 $\mu g \cdot ml^{-1}$, e) 50.0 $\mu g \cdot ml^{-1}$, when 16.0 $\mu g \cdot ml^{-1}$ hydrochlorothiazide used as divisor in methanol ($\Delta\lambda$: 8)

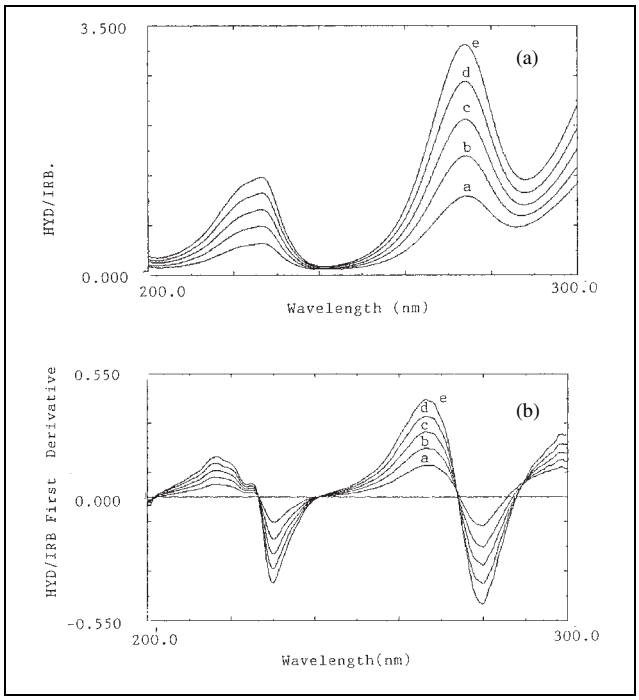

Fig. 3: Ratio spectra (a) and first derivative of the ratio spectra (b) of hydrochlorothiazide of a) 4.0 $\mu g \cdot ml^{-1}$, b) 9.0 $\mu g \cdot ml^{-1}$, c) 14.0 $\mu g \cdot ml^{-1}$, d) 19.0 $\mu g \cdot ml^{-1}$, e) 24.0 $\mu g \cdot ml^{-1}$, when 20.0 $\mu g \cdot ml^{-1}$ irbesartan used as divisor in methanol ($\Delta\lambda$: 8)

Table 4: Statistical analysis of calibration graphs of irbesartan and hydrochlorothiazide mixtures by the ratio first derivative spectrophotometry

Method					
Parameters	Irbesartan		Hydrochlorothiazide		
Wavelengths (nm)	237.8	247.8	230.0	266.1	279.4
Range ($\mu g \cdot ml^{-1}$)	10.0–50.0	10.0–50.0	4.0–24.0	4.0–24.0	4.0–24.0
Detection limits ($\mu g \cdot ml^{-1}$)	1.63	1.63	1.98	1.98	1.98
Regression equation (Y)[a]					
Slope (b)	1.15×10^{-3}	4.23×10^{-3}	9.34×10^{-3}	2.58×10^{-4}	6.45×10^{-4}
Std. dev. on slope (S_b)	2.89×10^{-4}	8.71×10^{-5}	7.36×10^{-6}	1.14×10^{-7}	6.63×10^{-7}
Intercept (a)	8.57×10^{-4}	9.22×10^{-5}	5.02×10^{-3}	3.86×10^{-3}	1.68×10^{-3}
Std. dev. on intercept (S_a)	1.96×10^{-4}	2.49×10^{-5}	8.60×10^{-6}	1.23×10^{-6}	3.74×10^{-6}
Std. error of estimation (S_e)	5.72×10^{-6}	3.98×10^{-6}	1.47×10^{-5}	7.95×10^{-5}	1.49×10^{-5}
Correlation coefficient (r)	0.9991	0.9984	0.9980	0.9998	0.9983
Rel. std. dev. (%)[b]	1.48	1.05	2.03	1.15	1.04
% Range of error[b] (% 95 confidence limit)	1.29	0.74	1.00	1.89	1.18

[a] $Y = a + bC$ where C is concentration in $\mu g \cdot ml^{-1}$ and Y in absorbance units. [b] Five replicate samples

simultaneous quantitative determination of irbesartan and hydrochlorothiazide were 238 and 266 nm. The influence of $\Delta\lambda$ on the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval; $\Delta\lambda = 8$ nm was considered as suitable. Under the described experimental conditions, the graphs obtained by plotting the derivative values of each drug in this mixture versus concentration, in the range stated in Table 4, showed linear relationships. The relative standard deviation values of the slope and intercepts of the calibration graphs indicated the high reproducibility of the proposed method. Conformity with Beer's law was evident in the concentration range of the final dilution cited in Table 4.

The correlation coefficients were 0.9991 and 0.9998, indicating good linearity. The relative standard deviations were found to be less than 1.48%, indicating reasonable precision. The detection limits (LOD) [25] were 1.14 $\mu g \cdot ml^{-1}$ for irbesartan and 1.08 $\mu g \cdot ml^{-1}$ for hydrochlorothiazide; while the quantification limits (LOQ) [26] were estimated to be 1.63 $\mu g \cdot ml^{-1}$ for irbesartan and 1.98 $\mu g \cdot ml^{-1}$ for hydrochlorothiazide. The method was successfully applied to the determination of these drugs in laboratory-prepared mixtures. The results are summarized in Table 5. The excipients (starch 10%, lactose 40%, talc 2% and magnesium strearate 1%) were added to the drug for recovery studies according to manufacturer's batch formula for per tablets. The new method was applied to the recovery of irbesartan and hydrochlorothiazide in three batches of commercial formulations, respectively. The results presented in Table 3 are in good agreement with the labelled content. All data represent the average of five determinations. Low values of relative standard deviation indicate very good precision.

Table 5: Recovery experiments obtained for different binary mixtures of irbesartan (IRB) and hydrochlorothiazide (HYD) by the ratio derivative spectrophotometry

Sample	Recovery (mean ± sd) %[a]					
	Irbesartan			Hydrochlorothiazide		
	Amount added ($\mu g \cdot ml^{-1}$)	Amount found ($\mu g \cdot ml^{-1}$)	Recovery (%)	Amount added ($\mu g \cdot ml^{-1}$)	Amount found ($\mu g \cdot ml^{-1}$)	Recovery (%)
	100.0			12.5	12.6	100.8
	125.0			12.5	12.2	97.6
	150.0			12.5	12.6	100.8
	200.0			12.5	12.1	96.8
	225.0			12.5	12.4	99.2
	150.0	149.9	99.9	7.5		
	150.0	150.1	100.1	10.0		
	150.0	148.5	99.0	15.0		
	150.0	148.9	99.3	17.5		
	150.0	148.9	99.3	20.0		

	$\overline{X}$ (%)	R.S.D (%)
HYD	98.6	1.17
IRB	99.0	1.00

2.3. Absorbance ratio method

Compensation technique and ratio first derivative spectrophotometry were used to test the absorbancy ratio method for binary mixtures. The zero-order (original) spectra of irbesartan and hydrochlorothiazide (Fig. 1) indicated that binary mixtures containing irbesartan and hydrochlorothiazide could be analyzed by applying the principles of the absorbance ratio method. By measuring absorbance values at 241 nm (λ_{max} for irbesartan), 272 nm (λ_{max} for hydrochlorothiazide) and 263 nm (isosbestic point) in the original spectra of the binary mixture in methanol, the analysis of the binary mixture containing irbesartan and hydrochlorothiazide was made by using the formulas explained in chapter 3.5.

A critical evaluation of the proposed method was performed by the statistical analysis of the experimental data. The slopes and y-intercepts are summarized in Table 6. In order to demonstrate the validity and applicability of the proposed methods, recovery studies were performed by

Table 6: Beer's law data and statistical analysis for the calibration graphs of irbesartan and hydrochlorothiazide with the absorbance ratio method

	Irbesartan	Hydrochlorothiazide
Solvent	Methanol	
λ_{max}	241 nm	272 nm
λ_{iso}	263 nm	
Concentration range for Beer's law compliance	10.0–50.0 $\mu g \cdot ml^{-1}$	4.0–24.0 $\mu g \cdot ml^{-1}$
$y = ax + b$	$y = 4.98x + 0.91$	$y = 5.73x + 0.48$

Table 7: Recovery experiments obtained for different binary mixtures of irbesartan (IRB) and hydrochlorothiazide (HYD) with the absorbance ratio method

Sample	Recovery (mean ± sd) %[a]					
	Irbesartan			Hydrochlorothiazide		
	Amount added ($\mu g \cdot ml^{-1}$)	Amount found ($\mu g \cdot ml^{-1}$)	Recovery (%)	Amount added ($\mu g \cdot ml^{-1}$)	Amount found ($\mu g ml^{-1}$)	Recovery (%)
	100.0			12.5	12.0	96.0
	125.0			12.5	12.5	100.0
	150.0			12.5	12.4	99.2
	200.0			12.5	12.6	100.8
	225.0			12.5	12.4	99.2
	150.0	150.1	100.1	7.5		
	150.0	150.5	100.3	10.0		
	150.0	147.9	98.6	15.0		
	150.0	148.9	99.3	17.5		
	150.0	149.9	99.9	20.0		

	$\overline{X}$ (%)	R.S.D (%)
HYD	99.5	1.50
IRB	99.7	1.42

analyzing synthetic mixtures of irbesartan and hydrochlorothiazide, with different composition ratios. The percentage recoveries of irbesartan and hydrochlorothiazide from spiked excipient are summarized in Table 7. The percentage recoveries and their relative standard deviations were found to be 99.7 and 1.42% for irbesartan and 99.5 and 1.50% for hydrochlorothiazide, respectively. Commercially available tablets containing mixture of irbesartan and hydrochlorothiazide in mixture were analysed by proposed methods and the results are presented in Table 3.

The proposed procedure was successfully applied to the determination of the studied compounds in pharmaceutical dosage forms. The methods described here are simple and accurate, requiring inexpensive reagents and could be used for rapid and reliable determinations of irbesartan and hydrochlorothiazide.

There is no official method for the simultaneous analysis of irbesartan and hydrochlorothiazide in binary mixture. Therefore, the absobancy ratio method was chosen as the analytical reference method. The compensation technique and ratio first derivative spectrophotometry were compared with absorbancy ratio method. The intercept values for compensation technique, ratio first derivative spectrophotometry and absorbancy ratio method were not statistically ($p < 0.05$) different from zero. No significant differences were found between the results obtained by the absorbances ratio method and the compensation technique and first derivative spectrophotometry, for same batch at the 95% confidence level (student's t-test).

The compensation technique and ratio derivative spectrophotometric methods are suitable techniques for the simultaneous determination of irbesartan and hydrochlorothiazide in multi-component formulations and without cross-interference. The ratio spectra derivative method and compensation techique are rapid, simple and sensitive, and results are available without solving equations or separation steps. In the ratio spectra derivative spectrophotometry, separate peaks and higher values of measurements can be obtained owing to the advantages of the selectivity of the divisor concentration. Higher values of measurements on the separate peak are an advantage for the ratio derivative spectrophotometry in comparison to the compensation technique. The absorbance ratio method is rapid, accurate and sensitive, and can be performed without solving equations or separation steps in the assay of irbesartan and hydrochlorothiazide in combination in pharmaceutical preparation.

Compensation technique and ratio derivative spectrophotometric method can be recommended for routine and quality control analysis of the investigated drugs in two-component pharmaceutical preparations for laboratories without sophisticated chromatographic instrumentation.

3. Experimental

3.1. Material

Hydrochlorothiazide and irbesartan were kindly donated by Sanofi-Doğu Pharm. Ind. All other chemicals were of analytical-reagent grade.

3.2. Apparatus

Spectrophotometric analysis was carried out on a Shimadzu 1601 double beam UV-Vis spectrophotometer with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with Shimadzu UVPC software and equipped with a Lexmark 1020 model printer.

3.3. Pharmaceutical preparation

A commercial pharmaceutical preparation, (Karvezide® tablet Sanofi Pharm. Ind., Turkey) was assayed. Its declared content was as follows: irbesartan 150.0, hydrochlorothiazide 12.5 mg/tablet.

3.4. Stock solutions and calibration graph

Stock solutions were prepared by dissolving irbesartan and hydrochlorothiazide in methanol to obtain a concentration of $1.0\ mg \cdot ml^{-1}$ for each compound. The standard solutions were prepared by dilution of the stock solutions in methanol to give concentration ranges in compensation method ratio derivative spectrophotometry and absorbance ratio method of $10.0–50.0\ \mu g \cdot ml^{-1}$ for irbesartan and $4.0–24.0\ \mu g \cdot ml^{-1}$ for hydrochlorothiazide, respectively.

3.5. Compensation technique

The second derivative spectra for each set of reference solutions with the appropriate solution were recorded. The second derivative maxima and minima ($^2D_{\lambda 1}/^2D_{\lambda 2}$), where done at the specified wavelengths (λ_1 and λ_2) as indicated in parentheses in Table 1.

A series of solutions containing different concentrations of pure drugs were prepared above and below that present in the binary mixture solution and placed in succession in the reference cell. The solution of the mixture (containing compounds irbesartan-hydrochlorothiazide) was placed in the sample cell. The second (^2D) absorption spectra of the solutions prepared were recorded and the corresponding ratio calculated (Table 1) in each instance followed by the calculated ratio for pure compound hydrochlorothiazide. The exact balance point (the ratio of the sample is equal to that of pure hydrochlorothiazide) at which the concentration of compound irbesartan in the sample solution is equal to that in the reference solution was determined. A similar procedure, following the same steps with solutions of pure compound hydrochlorothiazide in the reference cell, was performed to determine the concentration in the binary mixture at the balance point.

3.6. Absorbance ratio method

This method of analysis is based on the linear relationship between the absorbancy ratio of a binary mixture and the relative concentration of such a mixture. The quantification analysis of irbesartan and hydrochlorothiazide in a binary mixture were performed with the following equations :

$$C_1 = (Q_1 - b_1/a_1)\ (A_{iso}/a_{iso}) \times 10^3, \quad (1)$$

$$C_2 = (Q_2 - b_2/a_2)\ (A_{iso}/a_{iso}) \times 10^3 \quad (2)$$

where:

$Q_1 = A_1/A_{iso}$ for hydrochlorothiazide, $Q_2 = A_2/A_{iso}$ for irbesartan C_1 and C_2 = concentrations of the hydrochlorothiazide and irbesartan respectively A_{iso} = absorbance at isoabsorptive point ($\lambda_{iso} = 263$ nm), a_{iso} = absorptivity at isoabsorptive point = $A_{iso}/(C_1 + C_2)$, a_1 = slope of regression equation (Q_1 versus $C_1/(C_1 + C_2)$), a_2 = slope of regression equation (Q_2 versus $C_2/(C_1 + C_2)$), $b_{1,2}$ = intercept values of these regression equations, A_1 and A_2 denotes the absorbances of the mixture solution measured at λ_1 and λ_2 (272 nm and 241 nm).

References

1 Chang, S. Y.; Whigan, D. B.; Vachharajani, P. R.: J. Chromatogr. B **702**, 149 (1997)
2 Ghandour, M. A.; Kasm, E. A.; El-Haty, M. T.: Anal. Lett. **35**, 239 (2002)
3 Prieto, J. A.; Alonso, R. M.; Jimenez, R. M.: Electrophoresis **23**, 102 (2002)
4 Al-Majed, A. A.; Belal, F.; Al-Warthan, A. A.: Spectrosc. Lett. **34**, 211 (2001)
5 Prieto, J. A.; Akesolo, U.; Jimenez, R. M.: J. Chromatogr. A **916**, 279 (2001)
6 Kargosha, K.; Sarrafi, A. H. M.: J. Pharm. Biomed. Anal. **26**, 273 (2001)
7 El-Gindy, A.; Ashour, A.; Abdel-Fattah, L. et al.: J. Pharm. Biomed. Anal. **25**, 923 (2001)
8 Erk, N.: Anal. Lett. **35**, 283 (2002)
9 El-Gindy, A.; Ashour, A.; Abdel-Fattah, L. et al.: J. Pharm. Biomed. Anal. **25**, 171 (2001)
10 El-Gindy, A.; Ashour, A.; Abdel-Fattah, L. et al., J. Pharm. Biomed. Anal. **25**, 299 (2001)
11 Erk, N.: J. Pharm. Biomed. Anal. **24**, 603 (2001)
12 Luis, M. L.; Fraga, J. M. G.; Jimenez, A. I.; Arias, J. J.: Talanta, **53**, 761 (2001)
13 Sağlık, S.; Sağırlı, O.; Atmaca, S.; Ersoy, L.: Anal. Chi. Acta **427**, 253 (2001)
14 Lapa, R. A. S.; Lima, J. L. F. C.; Santos, J. L. M.: Anal. Chim. Acta **407**, 225 (2001)
15 Manna, L.; Valvo, L.; Alimonti, S.: Chromatographia **53**, S271 (2001)
16 Atay, O.; Tamer, U.; Arıkan, D.: Anal. Lett. **34**, 1153 (2001)
17 Carlucci, G.; Di Carlo, V.; Mazzeo, P.: Anal. Lett. **33**, 2491 (2000)
18 Carlucci, G.; Palumbo, G.; Mazzeo, P. et al.: J. Pharm. Biomed. Anal. **23**, 185 (2000)
19 Argekar A. P.; Sawant, J. G.: Anal. Lett. **33**, 869 (2000)
20 Belal, F.; Al-Zaagi, I. A.; Gadkariem, E. A. et al.: J. Pharm. Biomed. Anal. **24**, 335 (2001)
21 Hassib, S. T.; El-Sherif, Z. A.; El-Bagary, R. I. et al.: Anal. Lett. **33**, 3225 (2000)
22 Wahbi, A. A. M.; El-Yazbi, F. A.; Barary, M. H.; Sabri, S. M.: Analyst **117**, 785 (1992)
23 Salinas, F., Berzas Nevado, J. J.; Espinosa, M. A.: Talanta **37**, 347 (1990)
24 Berzas Nevado, J. J.; Guiberteau, C. C.; Salinas, F.: Talanta **39**, 2094 (1992)
25 Ng, L. L.: Reviewer Guidance: Validation of chromatographic Methods from the Centre for Drug Evaluation and Research, November, 1994
26 Shah, V. P.; Midha, K. K.; Dighe, S. et. al.: J. Pharm. Sci. **81**, 309 (1992)