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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(4): 722-733

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Septrofluorometric, Spectrophotometric and LC Determination of Irbesartan

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ABSTRACT

Simple, accurate and precise spectroflourimetric, spectrophotometric and LC methods have been developed and validated for the determination of irbesartan (IRB). The spectroflourimetric method depends on measuring the native fluorescence of IRB in the range of 1-6 µg.ml^{-1} (λem at 785 nm upon excitation at 250 nm) in 0.1N H_2SO_4. Spectrophotometric method represents a stability indicating assay for the determination of IRB in presence of its alkaline degradation product (IDP) in the range of 2.5-30 µg.ml^{-1}. This method was based on measuring the first derivative of ratio spectra at 236.5 nm. The LC method has been developed for the simultaneous determination of IRB and hydrochlorothiazide (HCZ) in presence of IDP in the range of 30 – 112.5 µg. ml^{1} and 2.5–9.375 µg. ml^{1} of IRB and HCZ, respectively. The analysis was conducted on Agilent zobrax ODS (C18) column, 5 µm particle size (4.6 x 250 mm), using ondansetrone hydrochloride as an internal standard and a mobile phase consisting of triethylamine: acetonitrile: 0.025 M potassium dihydrogen phosphate adjusted to pH (3) with o-phosphoric acid (0.15: 40: 60, v/v/v). Quantitation was achieved using UV detection at 269 nm at a flow rate maintained at 1 ml.min^{-1}. The results were statistically compared using one-way analysis of variance (ANOVA). The developed methods were satisfactorily applied to the analysis of the pharmaceutical formulations and proved to be specific and accurate for the quality control of the cited drugs in pharmaceutical dosage forms.

Keywords: Irbesartan; Hydrochlorothiazide; Spectrofluorometry; Spectrophotometry; Stability indicating; LC.

INTRODUCTION

Irbesartan (IRB), (Fig. 1a); Butyl-3-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]non-1-en-4-one is one of the Ag II receptor antagonists which are potentially safe and more tolerable than earlier classes of drugs used for the treatment of hypertension, diabetic nephropathy and heart failure [1]. A clinical study in hypertensive subjects has demonstrated that IRB effectively lowers blood pressure with a once daily dosage [1]. They are administered alone

or reinforced with the antihypertensive diuretic, Hydrochlorothiazide (HCZ) [2] (Fig. 1b). The USP official method for the determination of IRB is LC method on ODS (C18) column using a mobile phase consisting of a mixture of pH 3.2 phosphate buffer and acetonitrile (67:33, v/v) [3]. Several methods have been reported for the analysis of IRB in both pure and pharmaceutical dosage forms. These methods include LC [4-11], quantitative TLC [12,13], spectrophotometric [14-16], spectroflourimetric [16], HPLC-MS/MS [17], Voltametry [18] and capillary electrophoresis methods [19-21]. However, to the best of our knowledge, none of these methods was concerned with the analysis of IRB in the presence of its alkaline degradation product. Several chemical or physical factors can lead to the degradation of drugs [22], thus the aim of the present study was to develop more sensitive spectrofluorimetric and stability indicating spectrophotometric and LC methods for the assay of IRB with the application to pharmaceutical dosage forms that could be applied for drug quality control.

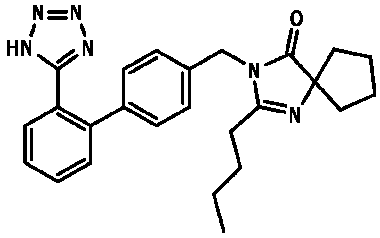

Fig. (1a).

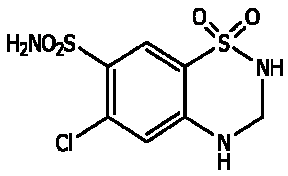

Fig. (1b).

Figure 1: Chemical structures of Irbesartan (a) and Hydrochlorothiazide (b).

EXPERIMENTAL SECTION

2.1. Instrumentation

A Jenway 6800 double beam ultraviolet/visible spectrometer, (U.K), connected to an IBM compatible computer with 1cm quartz cell and supported with Jenway flight deck software. Shimadzu RF-1501 Spectrofluorophotometer, (Japan). The LC system consisted of an Agilent 1100 liquid chromatograph (U.S.A) equipped with an isocratic pump G1310A, a manual injector G1328B with a 20 µl loop and a UV – visible variable wavelength detector. The separation was made on Agilent Zobrax ODS C18 column, 5 µm particle size (4.6 x 250 mm). The samples were injected (20 µl) with a 100 µl Agilent analytical syringe. A Soniclean120T ultrasonic processor (Australia) and a Jenway pH meter model 3505, Essex, (U.K) were also used.

2.2. Materials and reagents

IRB was kindly supplied by Bristol Mayer's Squib (Giza, Egypt) and with purity of 99.99% [1]. HCZ was kindly supplied by AstraZeneca (Cairo, Egypt) and it contained 100.64% [3]. OND was kindly supplied by GlaxoSmithkline (Cairo, Egypt) and certified to contain 99.95%. Water for LC was prepared by double glass distillation and filtration through 0.45-µm membrane filter. Methanol (LC grade) and acetonitrile (LC grade) were purchased from Fisher Scientific (Loughborough, Leicestershire, UK). Triethylamine (Sigma- Aldrich, Germany), potassium dihydrogen phosphate and sodium octane sulphonate (Oxford, India), *o*-phosphoric acid, sodium hydroxide, hydrochloric acid and chloroform (El-Nasr, Cairo, Egypt) were used. Membrane

filters 0.45µm from Teknokroma (Barcelona, Spain) were used. All other chemicals and reagents used were of analytical grade unless indicated otherwise.

2.3. Preparation of the mobile phase and adjusting the chromatographic conditions:

A mixture of triethylamine: acetonitrile: 0.025 M potassium dihydrogen phosphate adjusted to pH (3) with *o*-phosphoric acid: acetonitrile (0.15: 60: 40, *v/v/v*) was prepared. The LC separation and quantification were made on 4.6 x 250 mm (I.d) Agilent Zobrax ODS (5 µm particle size) C18 column. The mobile phase was filtered through 0.45 µm membrane filter and degassed using ultrasonic path. The flow rate was maintained at 1 $ml.min^{-1}$. The samples were injected (20 µl) with a 100 µl Agilent analytical syringe. All determinations were performed at ambient temperature. The system was equilibrated and saturated with the mobile phase for half an hour before the injection of the samples. Quantification was achieved with UV detection at 269 nm based on peak area. Data acquisition was performed on Agilent LC Chemstation software.

2.4. Preparation of IRB alkaline degradation product

Accurately weighed amount of IRB (0.2 gm) was refluxed in 50 ml 1 N sodium hydroxide for about 2 hours at 100°C. Then it was neutralized with 1N hydrochloric acid and extracted several times with 100 ml mixture of chloroform: diethyl ether (50:50, *v/v*). The extracts were combined and evaporated followed by crystallization of the residue from methanol. White crystals were formed. The crystals were filtered and dried. Test for complete degradation was done using TLC.

2.5. Preparation of standard solutions

2.5.1. Spectroflourimetric method

A solution of 0.5 $mg.ml^{-1}$ of IRB was prepared in methanol. Two ml aliquot of this solution was used to prepare a standard stock solution of 10 µg ml^{-1} of IRB in 0.1N H_2SO_4.

2.5.2. Spectrophotometric method

Two separate solutions of 0.5 $mg.ml^{-1}$ of IRB and IDP were prepared in methanol. Five ml aliquots from each solution were used to prepare standard stock solutions of 50 µg ml^{-1} of both IRB and IDP in 0.1N HCl.

2.5.3. LC method

Standard stock solutions of IRB, HCZ, IDP and OND of 0.75 mg ml^{-1}, 0.0625 mg ml^{-1}, 0.4 mg ml^{-1} and 0.2 mg ml^{-1}, respectively, were prepared in the mobile phase.

2.6. General procedures and calibration graghs

2.6.1. Spectroflourimetric method

Different aliquots (1 – 6 ml) of IRB standard stock solution equivalent to 10 – 60 µg were accurately measured and transferred into a set of 10 ml volumetric flasks and adjusted to volume with 0.1N H_2SO_4. The relative fluorescence intensities were measured at the specified excitation and emission wavelengths (λem at 785 nm with λex at 250 nm), then plotted against the corresponding concentrations and the regression parameters were computed.

2.6.2. Spectrophotometric method

Different aliquots (0.5 – 6 ml) of IRB standard stock solution equivalent to 25 – 300 µg were accurately measured and transferred into a set of 10 ml volumetric flasks and adjusted to volume

with 0.1N HCl. The absorption spectrum of each concentration of IRB was recorded against 0.1N HCl as a blank. The spectra of IRB were divided by the spectrum of 2.5 μg.ml^{-1} of IDP which is the chosen devisor. The first derivative of the ratio spectra were obtained using the following instrumental parameters: Δλ= 10, scaling factor = 5. The amplitudes at 236.5 nm were measured then plotted against the corresponding concentrations and the regression parameters were computed.

2.6.3. LC method

Accurately measured aliquots of IRB standard stock solution (0.4 – 1.5 ml) and HCZ standard stock solution (0.4 – 1.5 ml), equivalent to (30 – 112.5 μg) and (2.5 – 9.375 μg) of IRB and HCZ, respectively, were transferred into a series of 10 ml volumetric flasks. 1 ml aliquot of OND solution (internal standard) was added to each flask and the volume was adjusted with the mobile phase. Twenty micro liter of each flask was injected into the column. Each solution was prepared in triplicate. The chromatograms were recorded using the method parameters: Flow rate (1 ml.min^{-1}) and wave length (269 nm). The ratios (R) of the recorded AUPs of IRB and HCZ to that of OND were plotted versus the concentrations of IRB and HCZ (μg/ml) to obtain the calibration curves. The regression parameters were computed.

2.6.4. Stability indicating characteristics of the spectrophotometric method

Aliquots of IRB and IDP standard stock solutions equivalent to (50 - 200 μg) and (50 – 175 μg), respectively, were transferred into a series of 10 ml volumetric flasks. The volumes were adjusted with 0.1N HCl to prepare different laboratory prepared mixtures containing 17 – 58 % of the degradation product. The general procedure described under calibration was followed.

2.6.5. Stability indicating characteristics of the LC method

Aliquots of IRB, HCZ and IDP standard stock solutions equivalent to (0.3 – 1.125 mg), (25 – 93.75 μg) and (120 – 440 μg), respectively, were introduced into a series of 10 ml volumetric flasks. 1 ml aliquot of OND standard stock solution (internal standard) was added to each flask and the volume was adiusted with the mobile phase in order to prepare different mixtures containing 21 – 78 % of the degradation product relative to intact IRB. The general procedure described under calibration was followed.

2.7. Sample preparation

2.7.1. Spectroflourimetric and spectrophotometric methods

A quantity of the powdered Aprovel® 150 mg tablets equivalent to 25 mg IRB was extracted with 50 ml methanol and filtered. Further dilutions of the filtrate were made with 0.1N H_2SO_4 and 0.1N HCl to suit the spectroflourimetric and spectrophotmetric methods, respectively. The general procedures described under calibration were followed.

2.7.2. LC methods

An accurately weighed quantity of the powder Coaprovel® tablets equivalent to 75 mg IRB and 6.25 mg HCZ was extracted with 100 ml methanol and filtered. Further dilutions of the filtrate were made with mobile phase to suit the LC method. The general procedure described under calibration was followed.

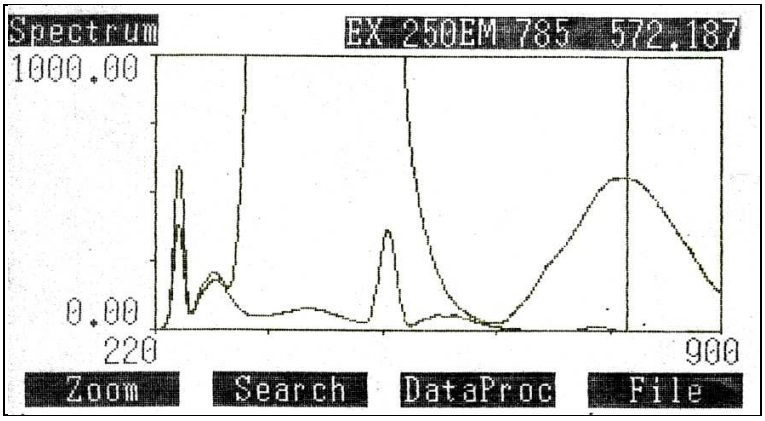

Figure 2: Excitation and emission spectrum of 5.5 µg ml^{-1} IRB in 0.1N H_2SO_4.

Table (1): Study the effect of different solvents on the fluorescence intensity of IRB

Solvent used	Amount of IRB (µg/ml)	Relative fluorescence of IRB at 785 nm
Methanol	5.0	141.585
Ethanol	5.0	9.821
0.1N HCl	5.0	593.519
0.1N H_2SO_4	5.0	659.291

Table (2): Results obtained by the proposed spectroflourimetric method for the determination of Irbesartan in the presence of its alkaline degradation product

Item	Spectrofluorometric method
λ_{max} excitation of measurements	250 nm
λ_{max} emission of measurements	785 nm
Range of linearity	1- 6 µg ml^{-1}
Regression equation	$F^*_{785\ nm} = 117.05C_{(\mu g.ml^{-1})} + 42.18$
Regression coefficient (r^2)	0.9993
LOD µg.ml^{-1}	0.027
LOQ µg.ml^{-1}	0.082
Standard deviation of slope (S_b)	1.577
Standard deviation of intercept (S_a)	6.142
Confidence limit of the slope	117.05 ± 0.22
Confidence limit of the intercept	42.18 ± 0.86
Standard error of the estimation	6.598
Results	
Drug in bulk	100.03 ± 1.37
Drug in dosage form (Aprovel® 150 mg)	95.95 ± 1.17
Drug added	100.04 ± 0.76

*F:Relative flourescence

RESULTS AND DISCUSSION

3.1. Spectroflourimetric method

IRB exhibits strong native fluorescence at 785 nm upon excitation at 250 nm (Fig. 2). This permits the development of a very sensitive method of assay for IRB in pure and pharmaceutical dosage forms. Different solvents such as methanol, ethanol and hydrochloric acid and sulfuric

acid were studied so as to give the best sensitivity and stability (Table 1). This study led to the described procedure. Due to the high sensitivity of the spectroflourimetric method, it can be used for determination of very low concentrations of IRB. Also, this method can be used for the determination of the drug in pharmaceutical dosage forms with no influence from excepients. Results of the proposed method are given in (Table 2).

3.2. Spectrophotometric method

The present work was conducted for the selective determination of IRB in presence of its alkaline degradation product. The UV absorption spectra of IRB and IDP in 0.1N HCl were recorded (Fig. 3). The spectra display considerable overlap, hence direct UV determination of IRB in the presence of IDP seems to be difficult. Derivative ratio spectrophotometric technique was able to resolve such interference with the application to its pharmaceutical dosage forms. The theory of derivative ratio spectrophotometry is based on the use of certain derivatives of the ratio spectra of the mixture and the division of the amplitudes at each wavelength by the absorption spectrum of a standard solution of one of the components. Derivative ratio spectrophotometric has been applied extensively to the simultaneous determination of substances with overlapping spectra as an economic alternative to LC methods [23]. In the present work, this technique was used to solve the problem of overlapping absorption spectra of IRB and IDP. IRB has been determined in the concentration range of 2.5–30 μg ml^{-1} in 0.1N HCl using the spectrum of 2.5 μg ml^{-1} of IDP as a divisor. In order to optimize the ratio derivative method that was developed, the influence of different variables was studied. These variables include solvent, divisor concentration and smoothing factor. The careful choice of the divisor and the working wavelengths were of great importance as it is affecting both sensitivity and selectivity; accordingly, different concentrations of the degradation product (2.5, 5, 7.5 and 10 μg ml^{-1}) were tried as divisors. The best result in terms of signal to noise ratio, sensitivity and repeatability was achieved upon using 2.5 μg.ml^{-1} of IDP (Fig. 4). The first and second derivatives of the ratio spectra were obtained using the following instrumental parameters: Δλ= 10 and scaling factor = 5. Calibration curve was constructed at 236.5 nm representing a linear relationship between the amplitudes and the corresponding concentrations. The regression equations listed in (Table 3) was computed. The results of the proposed method are given in (Table 3).

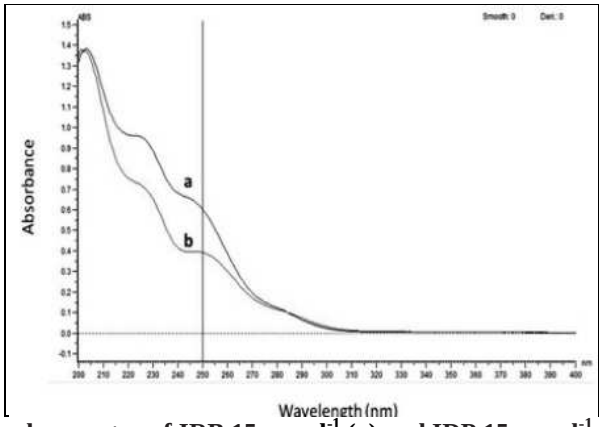

Figure 3: Zero order spectra of IRB 15 μg.ml^{-1} (a) and IDP 15 μg ml^{-1} (b) in 0.1N HCl.

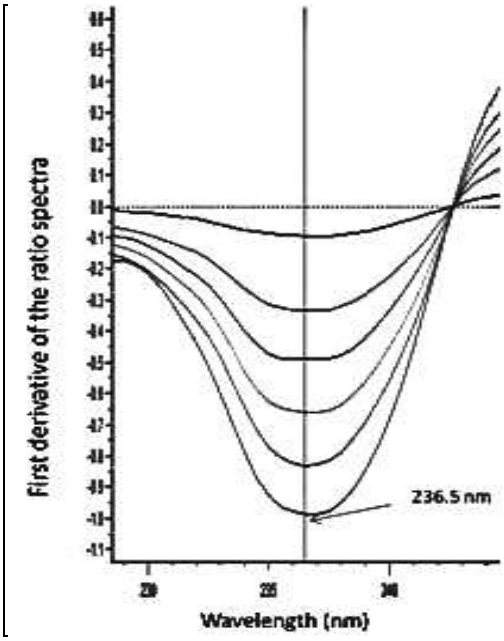

Figure 4: First derivative ratio spectra of IRB (2.5, 10, 15, 20, 25 and 30 µg/ml) using the spectrum of 2.5 µg/ml degradation product as a devisor.

Table (3): Results obtained by first derivative of the ratio spectra for the determination of Irbesartan

Item	First deivative
λ_{max} of measurements	236.5 nm
Range of linearity	2.5-30 µg ml^{-1}
Regression equation	$^{1}DD^{*}_{236.5} = 0.033\ C_{(\mu g.ml^{-1})} - 0.0009$
Regression coefficient (r^2)	0.9998
LOD µg.ml^{-1}	0.09
LOQ µg.ml^{-1}	0.28
Standard deviation of slope (S_b)	2.1×10^{-4}
Standard deviation of intercept (S_a)	4.1×10^{-3}
Confidence limit of the slope	$0.033 \pm 5.0 \times 10^{-5}$
Confidence limit of the intercept	$-0.0009 \pm 9.8 \times 10^{-4}$
Standard error of the estimation	4.7×10^{-3}
Results	
Drug in laboratory mixture	99.97 ± 1.16
Drug in dosage form(Aprovel® 150 mg)	98.76 ± 0.24
Drug added	100.72 ± 1.44

* $^{1}DD_{236.5\ nm}$*: peak amplitudes of the first derivatives ratio spectra at 236.5 nm.*

Method selectivity and stability indicating characteristics were checked by preparing and analyzing laboratory prepared mixtures at various concentrations within the linearity range. Satisfactory results were obtained and the mean percentage recovery of IRB in laboratory prepared mixtures was found to be 99.42 $\pm$ 1.21, indicating the high selectivity of the proposed method for the determination of IRB in the presence of up to 58% of the alkaline degradation product.

3.3. LC method

3.3.1. Method development

Method development consisted of the control of a number of parameters, related to the efficiency of the chromatographic system. Various reversed phase chromatographic columns and a series of aqueous mobile phases containing different organic modifiers at different ratios were tested to adjust the optimum conditions. The system suitability tests were used to verify that the conditions of the chromatographic system are adequate for the resolution and hence for the analysis [3]. A satisfactory separation was obtained with a mobile phase consisting of triethylamine: acetonitrile: 0.025 M potassium dihydrogen phosphate adjusted to pH (3) with *o*-phosphoric acid (0.15: 40: 60, *v/v/v*). The separation was improved with the relative increase of the percentage of the aqueous to organic phases. The ion pairing reagent, triethylamine was used in order to reduce tailing and improve resolution. The pH of the mobile phase was found to be critical in achieving the separation between all the compounds. pH (3±0.2) showed the best resolution. To improve the precision of the method and to compensate for small variabilities in the instrumentation performance, OND was used as an internal standard. Good separation of the cited compounds with good peaks' shapes and minimum retention times (< 14 min) were obtained with that mobile phase at a flow rate 1 ml min^{-1}. Quantitation was achieved with UV detection at 269 nm based on peak area. The retention times were 3.039, 4.810, 9.968 and 11.630 min for HCZ, OND, IDP and IRB, respectively, (Fig. 5).

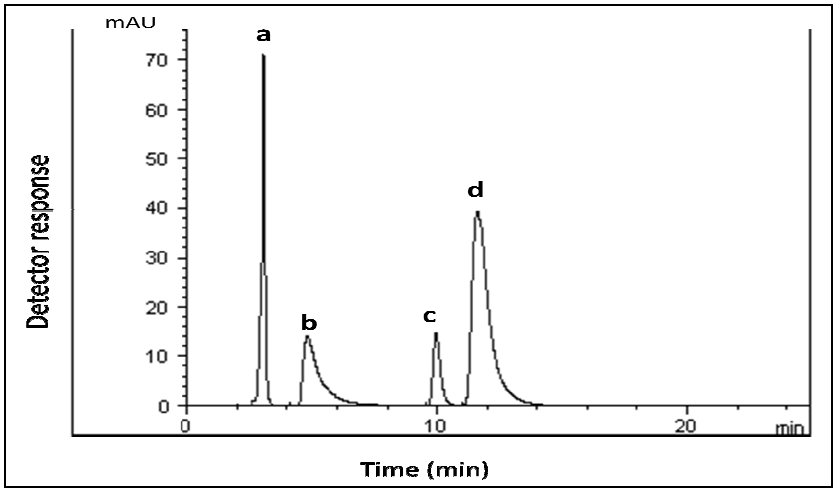

Figure 5: HPLC chromatogram of a mixture of: HCZ 9.375 µg/ml (t_r=3.039 min) (a), OND (internal standard) 20 µg/ml (t_r=4.810 min) (b), IDP 24 µg/ml (t_r=9.968 min) (c) and IRB 112.5 µg/ml (t_r=11.630 min) (d).

3.3.2. LC method validation

3.3.2.1. Linearity

The ratios (R) of the recorded AUPs of IRB and HCZ to that of OND were plotted against the corresponding concentrations (C) and linear relationships were obtained The regression equations listed in (Table 4) for each drug was computed. In this study, 6 concentrations for each compound were used. Ranges of linearity of each drug are mentioned in (Table 4). The linearity of the calibration curves were validated by the high values of correlation coefficients (Table 4). The analytical data of the calibration curves including standard deviations for the slope and intercept (S_b & S_a) are also summarized in (Table 4).

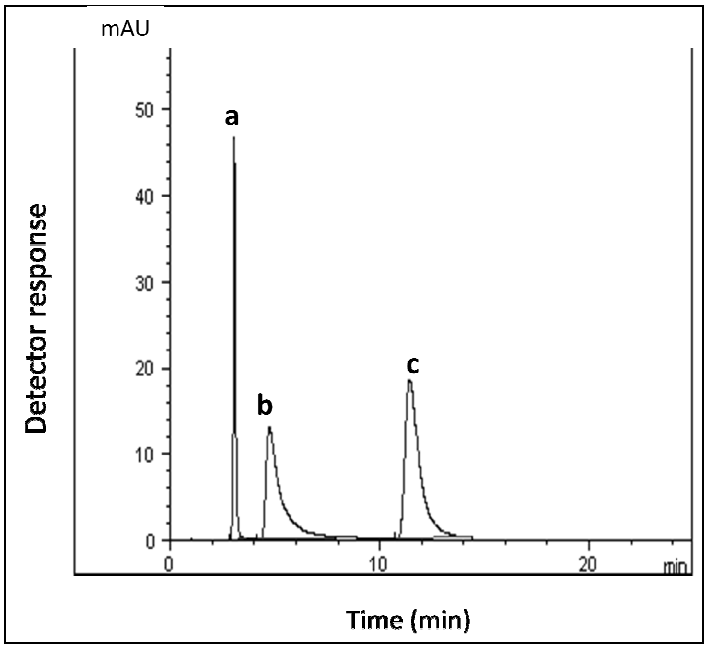

Figure 6: A typical LC chromatogram of 20 µL injector of a ternary mixture of HCZ (5 µg ml^{-1}) (a), OND (internal standard) (20 µg ml^{-1}) (b) and IRB (60 µg ml^{-1}) (c).

3.3.2.2. Precision

The precision was checked; within day (n= 3) and between days (n= 3) for three different concentrations at low, medium and high level of the standard curve. The relative standard deviations and the standard deviations of tablets were calculated to check the precision of the method (Table 4).

3.3.2.3. Accuracy

The accuracy of the method was tested by analyzing different solutions of the cited drugs at various concentration levels in their pharmaceutical dosage forms, (Fig. 6). The results were expressed as percent recoveries of the particular components in the samples. A possible matrix effect was taken into consideration. No blank matrix being available, the method of standard addition was used to ensure accuracy of the proposed method. This study was performed by the addition of known amounts of the studied drugs to a known concentration of the commercial pharmaceutical tablets. The resulting mixtures were analyzed and results obtained were shown in (Table 4) indicating good accuracy of the proposed methods.

3.3.2.4. Selectivity

The selectivity of the method was checked by analyzing IRB and HCZ in laboratory prepared ternary mixture with IDP. Good resolution and absence of interference between drugs being analyzed are shown in (Fig. 6). The mean percentage recoveries were found to be 99.92$\pm$ 1.21 and 100.36$\pm$ 0.77 in case of IRB and HCZ, respectively, indicating that the method is not affected by the presence of up to 80% of IRB degradation product. The results of system suitability are summarized in (Table 5). Moreover, the chromatograms of the samples were checked for the appearance of any extra peaks. No chromatographic interference from any of the excipients was found at the retention times of the examined drugs (Fig. 6). These results

demonstrate that there was no interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the method. Besides, good recoveries were obtained in the sample indicating specificity of the proposed method (Table 4).

3.3.2.5. Limit of detection and limit of quantification

Limit of detection (LOD) represents the concentration of analyte at S/N ratio of 3 and limit of quantification (LOQ) at S/N ratio of 10 were determined for the proposed method and results are given in (Table 4). According to ICH recommendations [24], the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits.

3.3.2.6. Robusteness

Variation of pH of the aqueous part with *o*-phosphoric acid by ± 0.2 and the organic strength of the mobile phase by 2% didn't have significant effect on chromatographic resolution in HPLC method.

3.4. Statistical analysis

The results of the proposed methods were compared with those of the reference methods. Statistical comparison of the results obtained by the three methods for the determination of IRB was carried out by "SPSS statistical package version 11". The significant difference between groups were tested by one way ANOVA (F-test) at p=0.05 as shown in (Table 6&7). The test ascertained that there was no significant difference between the results.

Table (4): Determination of IRB and HCZ in ternary mixture with IDP using RP-HPLC method

Item	IRB	HCZ
Retention time	11.93 min	3.14 min
Wavelength of detection	269 nm	269 nm
Range of linearity	30-112.5 µg mL^{-1}	2.5-9.375 µg mL^{-1}
LOD µg.ml^{-1}	2.87	0.27
LOQ µg.ml^{-1}	8.657	0.81
Regression equation	R^*_{IRB} = 0.0201 $C_{(\mu g.ml^{-1})}$ - 0.0070	R^{**}_{HCZ} = 0.0825 $C_{(\mu g.ml^{-1})}$ + 0.0409
correlation coefficient (r)	0.9996	0.9999
Standard deviation of slope (S_b)	2.02 x 10^{-4}	4.16 x 10^{-4}
Standard deviation of intercept (S_a)	1.49 x 10^{-2}	2.57 x 10^{-3}
Confidence limit of the slope	0.0201 ± 5.7x10^{-5}	0.0825 ± 1.9x10^{-4}
Confidence limit of the intercept	-0.007 ± 4.5x10^{-3}	0.0409 ±.9.5x10^{-4}
Standard error of the estimation	0.014	0.023
Intra-day		
%R.S.D.	0.40 – 0.61	0.44 – 1.82
Inter-day		
%R.S.D.	0.50 – 0.73	0.28 – 1.85
Results		
Drug in laboratory mixture	99.92 ± 1.21	100.36 ± 0.77
Drug in dosage form (Coprovel®)	94.69 ± 1.06	96.27 ± 1.05
Drug added	100.24 ± 1.39	100.48 ± 1.32

** R_{IRB}: Ratio of AUP of IRB at 269 nm / AUP of OND at 269 nm.*
*** R_{HCZ}: Ratio of AUP of HCZ at 269 nm / AUP of OND at 269 nm.*

Table (5): System suitability results of the proposed RP-LC method

Compound	N	R	T	t_R	% R.S.D. of Peak Area Ratio
HCZ	1265	---	0.47	---	1.83
OND	1137	2.15	0.45	1.56	---
IDP	1616	2.65	0.44	1.62	---
IRB	1412	2.27	0.41	1.39	1.91

(N: number of theoretical plates; R: resolution factor; T: tailing factor; t_R: relative retention time)

Table (6): Statistical comparison between the results of the developed methods and the reference method for the determination of Irbesartan

Statistical term	Reference Method [3]	Spectrofluorimetric method	Spectrophotmetric method	RP-HPLC method
Mean	100.15	100.03	99.97	99.92
S.D.±	1.31	1.37	1.16	1.21
S.E. ±	0.59	0.61	0.47	0.49
%RSD	1.31	1.37	1.16	1.21
n	5	5	6	6
V	1.72	1.88	1.35	1.46
F*		2.492		

**Calculated P-value= 0.075, tabulated F-value (at p=0.05) = 2.85.*

CONCLUSION

The proposed spectrofluorimetric, spectrophotometric and LC methods provide simple, accurate and reproducible quantitative analyses for the assay of IRB in bulk and dosage forms. The spectroflourimetric method has the greatest sensitivity. While the spectrophotometric and LC methods are stability indicating. The developed spectrophotometric method offered distinct advantages in simplicity, selectivity and sensitivity. The LC method is more specific than the spectroscopic methods. The LC method can also be used for the simultaneous determination of IRB and HCZ. Finally, the developed methods can be used for the quality control of the cited drugs in ordinary laboratories.

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