INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

APPLICATION OF A RP-HPLC METHOD FOR COMPARATIVE STUDY ON THE DEGRADATION BEHAVIOR OF TWO ANGIOTENSIN II RECEPTOR ANTAGONISTS, VALSARTAN AND LOSARTAN POTASSIUM

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ABSTRACT

Valsartan (VAL) and losartan potassium (LOS) are antihypertensive drugs, which are closely related in structure. Both drugs were subjected to stress conditions of hydrolysis (in acidic, neutral and alkaline media), oxidation (using hydrogen peroxide), dry heat and air. A reversed phase high performance liquid chromatographic method (RP-HPLC) was established by using acetonitrile: phosphate buffer (0.02M, pH 3.5), (60: 40 v/v) as a mobile phase at flow rate of 1.5 ml/ min and detection at 225 nm. Several factors were studied under the different experimental stress conditions. These factors include reagents concentrations, the applied temperature during stress testing and time of reaction. The degradation studies indicated that LOS is highly susceptible to oxidation, while VAL is very sensitive to acid hydrolysis and exhibits higher sensitivity to alkaline medium than LOS. The two drugs showed nearly the same stability under neutral hydrolysis. The RP-HPLC method was validated according to the ICH guidelines; the linearity range is 8-120 and 10-100 µg/mL for VAL and LOS, respectively; precision, accuracy, robustness, ruggedness, specificity were also evaluated. Thermal analysis of the two drugs was achieved in hot air oven and results show that valsartan is less thermally stable than losartan. The proposed RP-HPLC method is simple, accurate, reproducible, stability-indicating and suitable for routine determination of VAL or LOS in bulk and dosage forms.

Keywords: RP-HPLC; Stability indicating method; thermogravemetric; Valsartan.

INTRODUCTION

Valsartan (VAL) is designated as *N*-(1-oxopentyl)-*N*-{[2'-(1*H*-tetrazol-5-yl)[1,1'-bi-phenyl]-4-yl]methyl}-*L*-valine2 (Fig.1a). losartan potassium (LOS), a potassium salt of : (2-butyl-4-chloro-1-{[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1*H*-imidazol-5-yl) methanol (Fig.1b). Valsartan and losartan are non-peptide, orally active angiotensin II (Type AT_1) receptor antagonists employed in the management of essential hypertension[1]. Various methods have been reported for analysis of valsartan in pharmaceutical formulations alone or with other drugs in combinations, they include HPLC[2-6], HPTLC[7], capillary zone electrophoresis[8, 9] spectrophotometric[10-12]. Many analytical methods were reported for the analysis of losartan especially in combination with other drugs, they include HPLC[13-21], HPTLC[22], electrophoretic[23,24] and spectrophotometric[25-28] methods.

Forced degradation plays a key role not just in the development of stability-indicating methods, but also in providing useful information about the degradation pathways and degradation products that could form during manufacture and storage. The information thus obtained will facilitate pharmaceutical development in areas such as manufacturing, formulation development, synthesis of degradation products (needed for purity determination) and packaging. Thus, stability testing is used to improve the quality of drug product.

In the pre-clinical formulation stage, the selection of a stable drug product formula is the primary goal. This led us to study and compare the behavior of the two drugs valsartan and losartan in different mediums and conditions, using simple, sensitive, accurate and rapid RP-HPLC method.

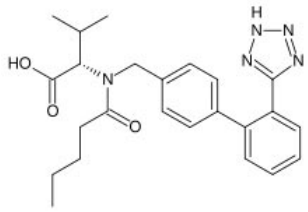

Fig. 1a: Chemical structure of valsartan

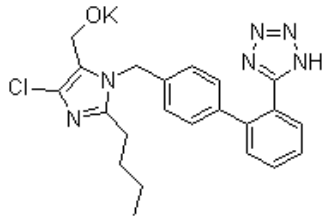

Fig. 1b: Chemical structure of losartan potassium

EXPERIMENTAL

MATERIALS

valsartan powder and its capsules, Disartan labeled to contain 80mg VAL per capsule were supplied from Global Napi (Egypt).

losartan powder and tablets, Kanzar labeled to contain 100 mg LOS per tablet were provided by Hikma company, Cairo- Egypt.

Acetonitrile and methanol, HPLC grade (rankem, India). All other chemicals are of analytical reagent grade. High pure water was prepared by using Millipore purification system.

Instrumentation and chromatographic conditions

The LC system, used for method development, forced degradation studies, and method validation was Agilent 1200 series liquid chromatographic RRHT (Rapid Resolution High Throughput) system comprising of binary pumps, column oven, autosampler and UV detector. The chromatographic column used was ACE C18 (250mm X 4.6mm X 5µ). The mobile phase consists of a mixture of 0.02 mM sodium dihydrogen orthophosphate (pH adjusted to 3.5 using orthophosphoric acid) and acetonitrile in 40:60 ratio. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.5 ml/min for 20 min. The column temperature was maintained at 40 °C. The eluate was monitored at 225 nm using UV detector and the injection volume was 20 µl. A shimadzu 1601 spectrophotometer with quartz cells of 1- cm optical path length and a Hanna Microprocessor HI 9321 with a combined glass-saturated calomel electrode were used.

Preparation of standard solutions

Standard stock solution (1mg/ml) of drug was prepared by dissolving 25 mg of valsartan or losartan in 5ml methanol or water, respectively. The solution was then ade up to volume in 25 ml volumetric flask with mobile phase.

Calibration curve (general procedure)

Calibration curves were prepared by taking appropriate aliquots of the standard stock solutions of the drug in different 10 ml volumetric flasks and the solutions were made up to volume with phosphate buffer pH 3.5 to give final concentrations of 8-120 µg/ml and 10-100 µg/ml for VAL and LOS, respectively. These standard solutions were analyzed in five replicates. The peak areas were plotted against concentration and the data was subjected to linear regression. Fig. 2a and Fig. 3a show standard chromatograms of 25 µg/ml valsartan and losartan, respectively.

Preparation of marketed formulations for assay

Ten tablets were weighed, finely powdered (or the content of 10 capsules for valsartan), and an accurately weighed sample of powdered tablets or capsules equivalent to 25 mg of losartan or valsartan was treated with few milliliters of mobile phase. The solution was then sonicated for 30 minutes, this solution was filtered through 0.45 µm filter paper and then diluted with mobile phase in a 25 ml volumetric flask to yield a starting concentration of 1 mg/ml.

Forced degradation studies

In order to determine whether the analytical method and assay were stability-indicating, valsartan and losartan active pharmaceutical ingredient (API) powder were stressed under various conditions to conduct forced degradation studies.

Acid, alkaline and neutral degradation

Forced degradation in acidic and alkaline media was performed by adding 20 ml of either HCl or NaOH (0.1, 0.5 or 1N) to 10 ml of the standard stock solution (1 mg/ml) in a 100 ml tightly capped conical flasks. The flasks were kept in dark in a water bath thermostated to 80° C up to

3h and then neutralized using NaOH or HCl of appropriate concentration then transferred to 100 - volumetric flasks and diluted to volume with phosphate buffer pH 3.5. Diluted solutions were prepared by appropriate dilution in the same solvent. Degradation under neutral condition was performed by adding 20 ml of distilled water instead of acid or base.

Oxidative degradation

20 ml of either 3.0 or 30.0 % hydrogen peroxide was added to 10 ml of standard stock solution then kept in dark at 40 ^{o} C in a thermostated water bath up to 24 h. The solution was then completed to volume with phosphate buffer pH 3.5 in a100 ml volumetric flask.

Thermal degradation

Thermal treatment was performed by exposing the drug to a controlled temperature oven at 60 ^{o}C for three days in tightly closed vials. The solution was then prepared in phosphate buffer pH 3.5 to achieve final concentration 100 µg/ml. The proposed HPLC method (general procedure) was then applied to the drugs kept under this condition and as well as under the same conditions but protected from light and heat (at room temperature, 35±2 ^{o}C and 75±5 % RH).

Stability on exposure to air

Effect of air was performed by keeping each of the drugs in Petri plates in a thin layer of 1mm thickness in dark, at room temperature (35±2 ^{o}C) and 75±5 % RH for one week. The proposed HPLC method was applied to the drugs kept under these conditions as well as under the same conditions but protected from air,

RESULTS AND DISCUSSION

HPLC method: Development and optimization

Method development and optimization

An isocratic method was found suitable to optimize the separation of major degradation products formed under various stress conditions. In the preparative chromatogram steps, UV overlain spectra of the two drugs and of all degradation solutions showed that valsartan, losartan and their degradation solutions absorbed appreciably at 225 nm. So, detection at this wavelength was selected for method development. The composition, pH, flow rate of the mobile phase and column temperature were changed to optimize the separation conditions. A mobile phase consisting of 0.02 mM NaH_2PO_4 buffer (pH 3.5) and acetonitrile (40:60 v/v), a flow rate of 1.5 ml/min and column temperature maintained at 40 ^{o}C was found to yield satisfactory retention time with sharp symmetrical peaks. The standard chromatogram of each drug (50 µg/ml) is shown in Fig. 2 a and Fig. 3 a.

Results of forced degradation studies

Forced degradation studies were performed for bulk drug, to provide an indication of the stability indicating property and specificity of the proposed method. This degradation is mainly observed in terms of loss of assay.

Acid and alkaline degradation

By keeping standard solution of valsartan or losartan in 0.1N, 0.5N and 1N at 80 ^{o} C up to 3h, about 12 or 8 % of valsartan or losartan was degraded in 0.1N HCl. Increasing the HCl concentration valsartan showed a higher sensitivity to the acid than losartan; since in 0.5 N and 1N HCl, VAL concentration decreased of 40 and about 68%, respectively, with appearance of three new additional peaks at 1.46, 1.81 and 4.45 min beside the drug peak at about 3 min; Losartan concentration decreased 15 and 19 % respectively; with appearance of only a small peak at retention time 1.46 min (Fig. 2b & Fig. 3b) in addition to the drug peak at about 2.6 min. At more drastic condition, when heating the drug with 2N HCl at 100 ^{o}C for 8 h, losartan lost about 28% of its concentration while valsartan lost about 78% of its concentration, which shows the great sensitivity of valsartan to HCl acid compared to losartan. When heated with 0.1N NaOH at 80 ^{o}C for 3 h no sufficient degradation was observed for both drugs. Treatment with 0.5 N NaOH at 80 ^{o}C for 3 h, 88% and 72% of losartan and valsartan remained, respectively; with appearance of only a new small peak at about 1.470 min for both drugs in addition to smaller one at 2.48 min for valsartan. By using 1N NaOH further degradation was observed for valsartan more than losartan (Fig.2c and Fig.3c).

Oxidative degradation

When valsartan or losartan standard solution was kept in 3% hydrogen peroxide up to 24 h at 40 ^{o}C no degradation was observed for valsartan while losartan degraded by about 6%. In 30% H_2O_2 when kept at 40^{o} C for up 24 h, losartan showed great sensitivity, it lost about 94% of its assay with appearance of many additional small peaks around the main peak of the drug while valsartan showed about 14 % decrease in its concentration (Fig.2d and Fig.3d). This result confirms the oxidizable nature of losartan which was confirmed by the previous work done by Zhao et al. [20] in which two main losartan oxidative degradates were

identified when losartan tablets were stored for three years at 40 ^{0}C /75% RH. They were formed by oxidation of hydroxyl group present in losartan (-CH-OH) to form aldehyde form(-CHO) or keto degradate (-C=O). Also, M.,Vidyawathi et al. in a pharmacokinetic study and due to presence of the hydroxyl group, they showed the tendency of losartan to form two oxidation degradates or active metabolites using fermentation techniques(carboxylic acid and 3'- hydroxy losartan metabolites) [29].

Stability in dissolving solvents and on exposure to air.

Losartan and valsartan were found to be nearly stable when kept in water at 80^{o}C for 3h, less than 2 % degradation was observed and when standard stock solution of valsartan in methanol or losartan in water was kept at room temperature (35±2^{o}C) for one weak, losartan showed 1.2 % decrease and the peak area of valsartan in methanol diminished by about 5%.

On exposure to open air, in dark at room temperature (35±2 ^{o}C) and 75± 5 % RH for one week, losartan concentration showed a decrease of about 4% while valsartan remained stable.

Thermal stability

Thermal stability of the two drugs was studied by exposing the drug to hot air (in oven at 60 ^{o}C) for three days. The proposed HPLC method (general procedure) was then applied to the drugs kept under this condition and as well as under the same conditions but protected from heat (ambient temperature). Results showed a decrease in valsartan concentration of about 5% and no change was observed for losartan (stress degradation results are summarized in table 1). In all cases, the proposed HPLC method effectively separates the drugs from their degradation products and thus can be used as stability-indicating.

Validation of the method (analytical parameters)

The method of analysis was validated as per the recommendations of ICH [30] for the parameters like linearity, Intra and inter-day precision, ruggedness, robustness, accuracy, limits of detection (LOD) and quantification (LOQ), and specificity.

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. Peak areas of valsartan and losartan were plotted against their respective concentrations and linear regression analysis performed on the resultant curve. The constructed calibration curves were linear over the concentration range of 8-120µg/ml and 10-100 µg/ml for VAL and LOS, respectively. Correlation coefficient (*n*=3) was found to be 0.999 and 0.998 for VAL and LOS, respectively. Regression line equation was found to be:

Y**=** -65.25+52.92 X and Y= 69.68+ 64.04 X for valsartan and losartan, respectively.

The limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve. LOD values for valsartan and losartan were found 2.11 µg/ml and 2.99 µg/ml, respectively, and limits of quantification were found 6.40 µg/ml and 9.07 µg/ml respectively.

Precision

Intra-day precision of the method was studied by calculating the relative standard deviation (RSD %) of the peak area for six determinations of solutions of pure drug at three concentrations 20, 50, and 80 µg/ml. All determinations were made on the same day and under the same experimental conditions. Inter-day precision was assessed by injecting the same three concentrations over three consecutive days, results (Table 2) indicate acceptable level of inter-day and intra- day precision. The ruggedness of the method was assessed by comparison of the intra- and inter-day assay results for valsartan or losartan undertaken by two analysts. The % RSD values for intra- and inter-day assays of the drug performed in the same laboratory by the two analysts did not exceed more than 2%, thus indicating the ruggedness of the method.

Recovery (standard addition method)

Recovery studies by the standard addition method were performed. For this reason, a known amount of the pure drug (50 µg) which was previously analyzed, were spiked with 30, 50 or 75 µg losartan or valsartan standard solutions, and the mixtures prepared in triplicate, were analyzed. The values of recovery % and RSD % listed in table 3 indicate that the method is accurate.

Specificity

The results from the stress studies indicated that the method was highly specific for VAL and LOS. The degradation products were completely

distinguishable from the parent compound. Based on peak purity of VAL and LOS, every degradation sample showed that the peaks were homogenous and no co eluting peaks were observed indicating that the method was stability indicating and specific.

ASSAY

The validated method was applied to the determination of valsartan and losartan in commercially available disartan capsules and kanzar tablets. The results of the assay (*n* =5) undertaken yielded 99.73% and 100.40 (% RSD less than 2%) of label claim for valsartan and losartan, respectively (Table 4). The results of the assay indicate that the method is selective for the analysis of valsartan or losartan without interference from the excipients used to formulate and produce these capsules or tablets.

CONCLUSION

A simple, rapid, accurate, and precise stability-indicating HPLC analytical method has been developed and validated for the routine analysis of valsartan or losartan in their formulations without any interference from excipients. The results of stress testing reveal that the method is selective and stability indicating. The proposed method has the ability to separate the drug from their degradation products, and can be applied to the analysis of samples obtained during accelerated stability experiments. Results showed that LOS is highly susceptible to oxidation. Consequently, adequate air protection should be adopted for its packaging, storage and handling; while VAL is very sensitive to acid hydrolysis and exhibits higher sensitivity to alkaline medium than LOS. The two drugs showed nearly the same stability under neutral hydrolysis.

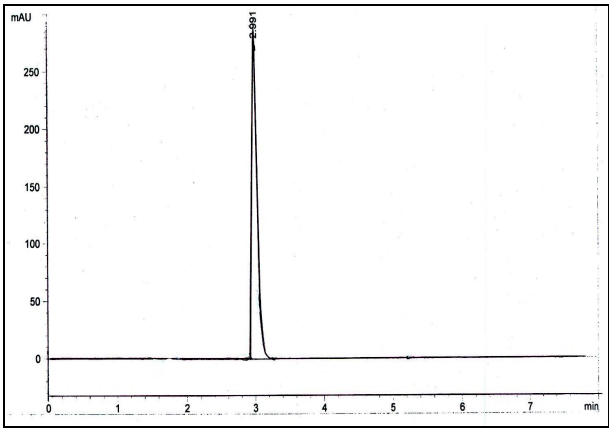

Fig. 2a: Chromatogram of Valsartan standard 25 µg/ml

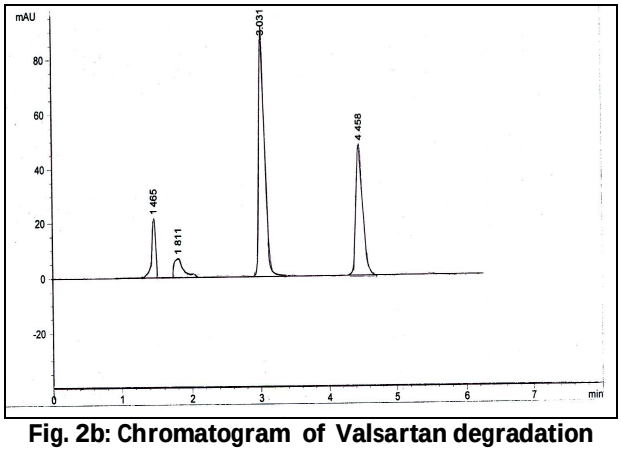

Fig. 2b: Chromatogram of Valsartan degradation product 25 µg/ml in 1N HCl

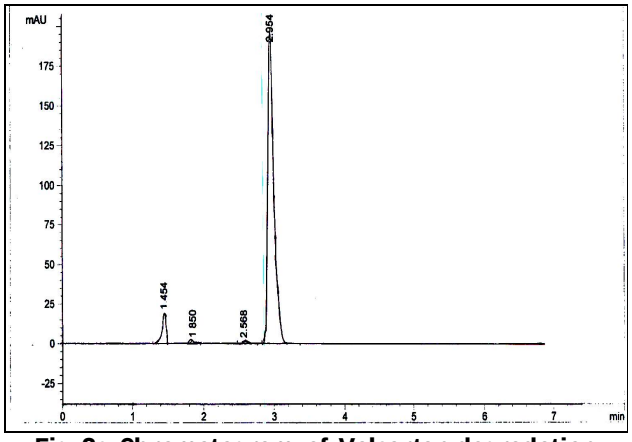

Fig. 2c: Chromatogram of Valsartan degradation product 25 µg/ml in 1N NaOH

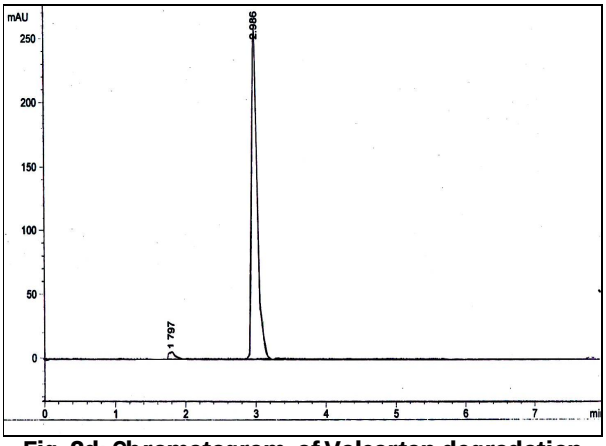

Fig. 2d: Chromatogram of Valsartan degradation product 25 µg/ml in 30% H_2O_2

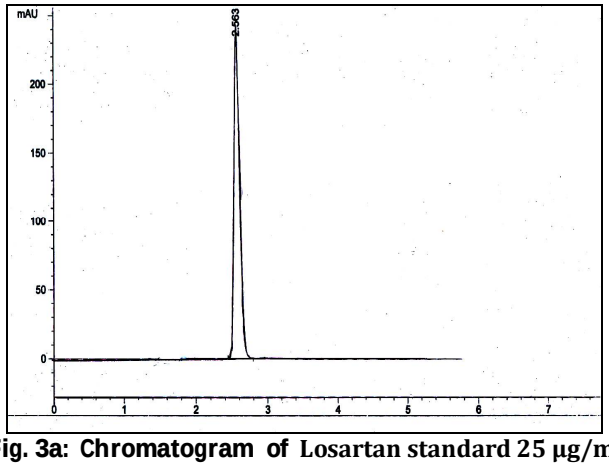

Fig. 3a: Chromatogram of Losartan standard 25 µg/ml

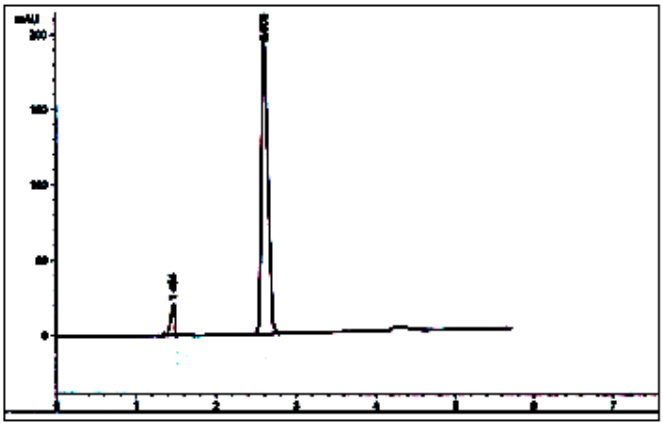

Fig. 3b: Los Chromatogram of artan degradation product 25 µg/ml in 1N HCl

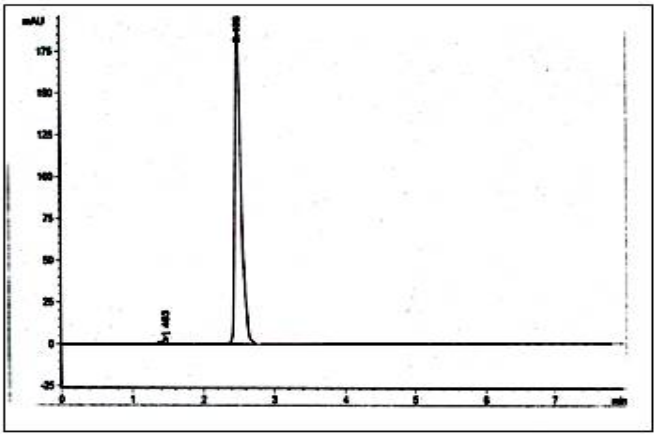

Fig. 3c: Chromatogram of Losartan degradation product 25 µg/ml in 1N NaOH

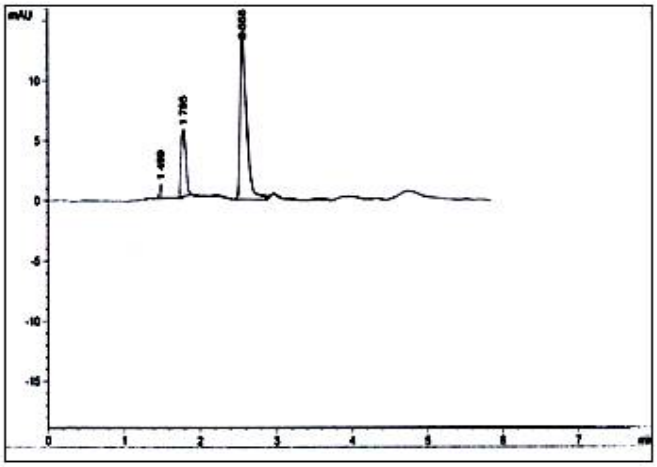

Fig. 3d: Chromatogram of Losartan degradation product 25 µg/ml in 30% H_2O_2

Table 1: Results of the Stress Degradation Tests of valsartan and losartan Using Different Conditions

Agent	Exposure time(h)	Heating temperature (°C)	Assay %	
			valsartan	losartan
HCl				
0.1N	3	80	88.0	92.0
0.5N	3	80	60.0	85.0
1N	3	80	32.3	81.4
2N	8	80	22.0	72.0
NaOH				
0.1N	3	80	96.0	98.0
0.5N	3	80	72.0	88.0
1N	3	80	59.4	87.0
H_2O_2				
3%	24	40	99.3	94.1
30%	24	40	86.1	6.2
Solvents				
H_2O	3	80	98.7	98.0
	7 days	Room temp 35±2	-	98.8
Methanol	7 days	Room temp 35±2	95.0	-
Open air 75± 5 % RH	7 days	Room temp 35±2	99.8	95.9
oven	3 days	60	95.3	99.9

Table 2: Intra-day and inter-day precision

Conc (µg/ml)	Intra-day precision Mean area± SD RSD %		inter-day precision Mean area± SD RSD %	
	VAL	LOS	VAL	LOS
30	29.87± 0.33 1.10	29.98±0.40 1.33	29.93±0.49 1.64	29.82±0.35 1.17
50	50.14±0.45 0.90	50.29±0.63 1.25	49.98±0.73 1.46	50.12±0.54 1.08
80	80.16±0.87 1.08	80.12±0.50 0.62	79.87±1.11 1.39	80.19±0.67 0.83

Table 3: Results from recovery studies with valsartan and losartan by standard addition method

drug	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml) mean± SD	RSD %	Standard analytical error (SAE)
Valsartan	50	30	29.99±0.41	1.36	0.24
		50	50.12±0.32	0.64	0.18
		75	74.95±0.51	0.68	0.29
Losartan	50	30	30.02±0.44	0.145	0.25
		50	49.96±0.25	0.5	0.15
		75	75.10±0.34	0.45	0.2

Table 4: Assay results for determination of valsartan and losartan in pharmaceutical formulations

Generic name	Brand name	Recovery(%) ± SD* RSD %	
		Proposed method	Reported method
Valsartan	Disartan capsules 80mg/capsule	99.73 ± 0.29 0.29	99.51± 0.63 0.64
Losartan	Kanzar tablets 100mg/tablet	100.40 ± 0.64 0.63	99.87±0.66 0.66

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