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STABILITY-INDICATING HPLC METHOD FOR DETERMINATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN PURE AND IN TABLET COMBINATION FORMS

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ABSTRACT

A simple, sensitive and accurate stability-indicating HPLC method has been developed and validated for determination of valsartan (VAL) and hydrochlorothiazide (HCT) in pure and in combination tablet forms. The Chromatographic separation was achieved within 10.0 min on Hypersil BDS C18column (250 x 4.6 mm, 5 µm particle sizes). The mobile phase contains a mixture of0.05 M phosphate buffer pH 3.5 and acetonitrile in ratio (50: 50). The isocratic RP- HPLC was investigated to separate the drugs from their stressed degradation products. The flow rate was 1mL/min and the column temperature was maintained at 40°C. VAL and HCT were subjected to stress degradation conditions of hydrolysis (acid and base), oxidation, thermal degradation at 80°C for 2 hours and photolytic degradation. Stressed samples were analyzed by the developed procedure. The described method shows excellent linearity over a range of 16.0 – 112.0 µg/mL, 2.5- 17.5 µg/mL for VAL and HCT, respectively and the correlation coefficients was 0.9996 for both VAL and HCT. The limit of detection was 4.6µg/mL and 0.72µg/mL while limit of quantitation was 15.3µg/mL and 2.1µg/mL for VAL and HCT, respectively. The suggested method was successfully applied for the analysis of the two drugs combination in tablet dosage form. The application of the proposed method was extended to stability studies of VAL and HCT after exposure to different forced degradation conditions according to ICH guidelines. Moreover, the method was validated for linearity, accuracy, precision, and robustness.

KEYWORDS

Stability Indicating, HPLC, Valsartan and Hydrochlorothiazide.

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INTRODUCTION

Valsartan, (S)-N-valeryl-N-[2′-(1H-tetrazol-5-yl) biphenyl-4-yl) methyl] valine, is a potent, highly selective, orally active, specific angiotensin II receptor antagonist used as a hypotensive drug, while Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H-1,2, 4-benzothiadiazine-7-sulphonamide-1, 1-dioxi-de, is a diuretic drug[1-7]. The chemical structures for both drugs are shown in Figure No.1. Stability testing is an important part of the process of drug product development. The drug product in a

stability test sample needs to bedetermined using a stability indicating method, as recommended by ICHguidelines[8]. Reviewing the literature revealed that several methods were developed for the determination of VAL and HCT; such as first derivative and second derivative.

Spectrophotometry[9,10], capillary electrophoresis[11-13], stripping voltammetric[14], TLC-densitometry[15,16], flow injection analysis[17], spectrofluorimetry[18-21], HPLC[22-28], liquid chromatography mass spectrometry (LC- MS)[29-33] and potentiometry[34]. The present study describes the development and validation of RP-HPLC method for stability evaluation and quantitative determination of valsartan and hydrochlorothiazide, which could be applied for the quantitative determination of the studied drugs combination in bulk powder and in tablet forms.

EXPERIMENTAL

Instruments and chromatographic conditions

Agilent 1200 (USA) HPLC system was used for analysis, the system equipped with quaternary pump (DIVAC 2.2), variable volume auto sampler, variable wavelength detector and thermostatted column compartment (TCC) which controls the temperature between 10°C below ambient and up to 100°C. The TCC is Hypersil BDS C18column (250 x 4.6 mm, 5 µm particle sizes) was used as stationary phase. The mobile phase composition used was a mixture of0.05 M phosphate buffer of pH 3.5 as aqueous solvent and acetonitrile as organic solvent in ratio (50:50). The best compromise between adequate resolution and reasonable retention times was achieved by using an isocratic system. The mobile phase was filtered using 0.45 µm membrane filters (Millipore, Cork, Ireland) and degassed using a Prominence degasser DGU-20A5.A Consort NV P-901 calibrated pH–Meter (Belgium) was used for pH measurements. Camag UV-Lamp (S/N 29000), dual wavelength (254/336), 2 x 8W (Muttenz, Switzerland) was used in the photo-stability study.

Materials and reagents

All the Reagents used were of Analytical Reagent grade and the solvents were of HPLC grade. High purity water was obtained by filtration of distilled water through 0.45 µm membrane filter (Millipore, Cork, Ireland) and was used throughout the study. Valsartan and hydrochlorothiazide were kindly provided by Global Napi, Cairo, Egypt. Their purity was found to be 98% and 90% respectively. Co-vasotec®80 mg tablets, labeled to contain 80 mg of valsartan and 12.5 mg of hydrochlorothiazide/tablet, products of Egyptian International Pharmaceutical Industries Company (EIPICO) (10th Ramadan, Egypt), were purchased from local pharmacy. The HPLC grade acetonitrile, methanol and analytical grade Orthophosphoric acid, potassium dihydrogen phosphate, supplied from Merck, Darmstadt, Germany. Water was doubly distilled and Sodium hydroxide (NaOH), hydrogenperoxide (H_2O_2), hydrochloric acid (HCL); were all obtained from El-Nasr Co. (ADWIC; Egypt).

General procedures

Preparation of stock and standard working solutions

12.5 mg of Hydrochlorothiazide and 80 mg of valsartan was transferred to a 250 mL volumetric flask, added 100 mL of mobile phase, sonicated for 15 min, diluted with methanol to 250 mL and mixed, 10 mL of this solution was transferred to a 50 mL volumetric flask, diluted with methanol to 50 mL.

Construction of the calibration graph

Accurately measured aliquots of standard solution ranging from 16.0- 112.0 µg/mL and 2.5- 17.5 µg/mL for VAL and HCT, respectively, were transferred into a series of 10 mL volumetric flasks. The volumes were completed to the mark with the mobile phase. Aliquots of 10 µL were injected (triplicate) and eluted with the mobile phase under the optimum chromatographic conditions. Detection was performed at wavelength 220 nm. The peak area versus the final concentration of the drug in µg/mL was plotted. Alternatively, the corresponding regression equation was derived.

Application of the proposed method to the determination of VAL and HCT combination in tablets

Twenty tablets of Co-vasotec® were weighted and grinded to a fine, uniform size powder. An

accurately weighted amount of the powder corresponding to 80.0 mg of VAL and 12.5mg of HCT were transferred into 250.0 mL volumetric flask, 30.0 mL of methanol were added. The contents of the flask were sonicated for 15 min, and completed to the volume with the mobile phase. Transfer 10 mL of this solution to a 50 mL volumetric flask, dilute with diluent to volume, and mix. Filter through a 0.45µm membrane filter. Aliquots containing suitable concentrations of the studied drugs were analyzed.

Procedures for forced degradation

Acidicdegradation

1.0 mL of stock solution (containing 0.064 mg of VAL and 0.01 mg of HCT) was transferred into 10.0 mL conical flask; 1.0 mL of 0.1 N HCL solutions was added. The solution was heated under reflux in boiling water bath for 2 hours. The content of the flask was cooled; neutralized to pH 7.0 with 0.01 N NaOH. Suitable aliquot of resultant degraded sample were withdrawn and subjected to analysis after suitable dilution with methanol.

Alkaline degradation

1.0 mL of stock solution (containing 0.064 mg of VAL and 0.01 mg of HCT) was transferred into 10.0 mL conical flasks; 1.0 mL of 0.1 N NaOH solutions was added. The solutions were heated under reflux in boiling water bath for 2 hours. The content of the flask were cooled; neutralized to pH 7.0 with 0.01 N HCL. Suitable aliquot of resultant degraded sample was withdrawn and subjected to analysis after suitable dilution with methanol.

Oxidative degradation

1.0 mL of stock solution (containing 0.064 mg of VAL and 0.01 mg of HCT) was transferred into 10.0 mL conical flasks; 1.0 mL aliquots of 30% H_2O_2 solutions were added. The solution was heated under reflux in boiling water bath at 80^{o}C for 2 hour. Suitable aliquot of resultant degraded sample was withdrawn and subjected to analysis after suitable dilution with methanol.

Thermal degradation

1.0 mL of stock solution (containing 0.064 mg of VAL and 0.01 mg of HCT) was transferred into 10.0 mL conical flasks and complete to the mark with doubly distilled water. The solution was heated under reflux in boiling water bath at 80^{o}C for 2 hour. Suitable aliquot of resultant degraded sample was withdrawn and subjected to analysis after suitable dilution with methanol.

Photolytic degradation

1.0 mL of stock solution (containing 0.064 mg of VAL and 0.01 mg of HCT) was transferred into 10.0 mL conical flask. The flask was exposed to UV-lamp at a wavelength of 254 nm at a distance of 15.0 cm placed in a wooden cabinet for 48 hours. At the specified time, the solution was removed from light source. Suitable aliquot of resultant degraded sample was withdrawn and subjected to analysis after suitable dilution with methanol.

RESULTS AND DISCUSSION

The optimum chromatographic performance were achieved when using Hypersil BDS C18column (250 x 4.6 mm, 5 µm particle sizes), isocratic mobile phase composed of 0.05 M phosphate buffer of pH 3.5 as aqueous solvent and acetonitrile as organic solvent in ratio (50:50), column temperature 40°C, detection wavelength 220 nm and flow rate 1 mL/min. Under the optimized conditions Valsartan was separated within 3.4 minute while hydrochlorothiazide was separated within 1.2 minute as shown in Figure No.2.

Degradation behavior of the investigated drugs

Acidic degradation

Valsartan is less susceptible to acidic hydrolysis. The drug on refluxing in 0.1N HCL resulted in formation of one degradation product for 2 hour in water bath. The degradation was about 10%, while, hydrochlorothiazide in the same condition give degradation of about 30%, as shown in Figure No.3 and Sheme No.1.

Alkaline degradation

Valsartan is stable to alkaline hydrolysis. After 2 hours; drug degraded by more than 60 % but hydrochlorothiazide degraded not more than 3%, as shown in Figure No.4 and Scheme No.2.

Valsartan was susceptible to oxidative degradation studies. Greater than 20 % of drug was degraded after 2 hours, while, hydrochlorothiazide was degraded in large amount after exposure to 30 %

H_2O_2 for 2 hours in boiling water bath, as shown in Figure No.5 and Scheme No.3.

Thermal degradation

Valsartan was susceptible to thermal degradation studies. More than 20 % of the drug was degraded after 2 hours, while, hydrochlorothiazide degraded in large amount after 2 hours in boiling water bath, as shown in Figure No.6.

Valsartan was found to be stable under photolytic degradation conditions. No degradation product peaks were observed on exposure of drug solution to UV-lamp at a wavelength of 254 nm for 48 hours, while hydrochlorothiazide degraded about 1%, as shown in Figure No.7.

Method validation

The validity of the proposed method was tested regarding linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, robustness and selectivity. The validity of the proposed method was tested regarding according to ICH recommendation[8].

Linearity and Range

Several concentrations of VAL and HCT solutions ranging from 16.0 to 112.0 µg/mL and 2.5 to 17.5 µg/mL for VAL and HCT, respectively, in ratio 6.4:1 were analyzed. The calibration graph of the peak area against concentration proved linearity in the range of 16.0 to 112.0 µg/mL and 2.5 to 17.5 µg/mL for VAL and HCT, respectively, while determination coefficient (R^2) was 0.9996 for both drugs, as shown in Table No.1.

Limit of detection and quantification

Limit of detection (LOD) defined as the injected quantity S/N ratio of 3 (in terms of peak height), were found to be 4.6 and 0.72 µg/mL for VAL and HCT, respectively. Limits of quantitation (LOQ) defined as the injected quantity giving S/N ratio of 10 (in terms of peak height), was found to be 15.3 and 2.1 µg/mL for VAL and HCT respectively. Results of the analysis are given in Table No.1.

Precision and repeatability of the method

Intra-day precision was assessed through replicate analysis of three concentrations of the studied drug on three successive times within the same day while inter-day precision were analyzed in triplicate on three consecutive days at 100% of the test concentration and percentage RSD were calculated. The results indicated high intra- and inter-day precisions as shown in Table No.2 and 3. Repeatability was investigated by injecting 6 determinations at 100% of the test concentration and percentages RSD were calculated. The RSD were found to be very small indicating reasonable repeatability and intermediate precision of the proposed method.

Accuracy

Results of the analysis were compared statistically to a reported HPLC method for the determination of mixture of VAL and HCT, applying the student (t-test) and variance ratio test (F- test). The results obtained were in good agreement with those obtained using the reported method as shown in Table No.4.

Robustness of the method

Robustness was performed by small change in the chromatographic conditions. The most important parameter to be studied was the resolution factor between the two peaks of VAL and HCT, as can be seen in Table No.5, good values of the resolution factors were obtained for all these variations, indicating good robustness of the proposed LC method.

Specificity of the method

The specificity of the assay was determined by the complete chromatographic separation of VAL and HCT peaks from their degradation product peaks generated under various stress conditions. The results indicated that the excipients in the tablets did not interfere with the determination of the drugs.

Analysis of valsartan and hydrochlorothiazide in tablet forms

The proposed method was successfully applied to determine VAL and HCT combination tablet form (Co-vasotec®). Four replicate determinations were performed. Satisfactory results were obtained for each compound in good agreement with label claims, as shown in Table No.6.

Table No.1: Linearity and calibration parameters data for the stability indicating chromatographic method of valsartan and hydrochlorothiazide mixture

S.No	Drug	Valsartan	Hydrochlorothiazide
1	Linearity range (µg/mL)	16.0 - 112.0	2.5 - 17.5
2	Slope	59.888	115.01
3	Intercept	-0.0896	-0.3746
4	Correlation coefficient *	0.9996	0.9996
5	Limit of detection (µg/mL)	4.6	0.72
6	Limit of quantitation (µg/mL)	15.3	2.1

*Linearity range starts from 16.0 µg/mL to achieve the ratio (6.4:1) as present in the tablet

Table No.2: Reproducibility and inter-day precision for the stability indicating chromatographic method of valsartan and hydrochlorothiazide mixture

S.No	Valsartan			Hydrochlorothiazide		
	Conc. Taken (µg/mL)	*mean ± SD	% RSD	Conc. Taken (µg/mL)	*mean ± SD	% RSD
1	32.0	32.076±0.161	0.503	5.0	4.990±0.0406	0.759
2	64.0	64.012±0.115	0.180	10.0	10.045±0.1224	1.219
3	96.0	95.865±0.309	0.322	15.0	15.017±0.0861	0.573

* Average of three determinations

Table No.3: Reproducibility and intra-day precision for the stability indicating chromatographic method of valsartan and hydrochlorothiazide mixture

S.No	Valsartan			Hydrochlorothiazide		
	Conc. Taken (µg/mL)	*mean ± SD	% RSD	Conc. Taken (µg/mL)	*mean ± SD	% RSD
1	32.0	32.141±0.225	0.700	5.0	4.978±0.0378	0.759
2	64.0	63.759±0.162	0.255	10.0	10.048±0.1207	1.201
3	96.0	96.070±0.280	0.292	15.0	14.999±0.077	0.513

* Average of five determinations

Table No.4: Statistical analysis of results obtained by the proposed stability indicating chromatographic method of VAL and HCT mixture compared with reported method

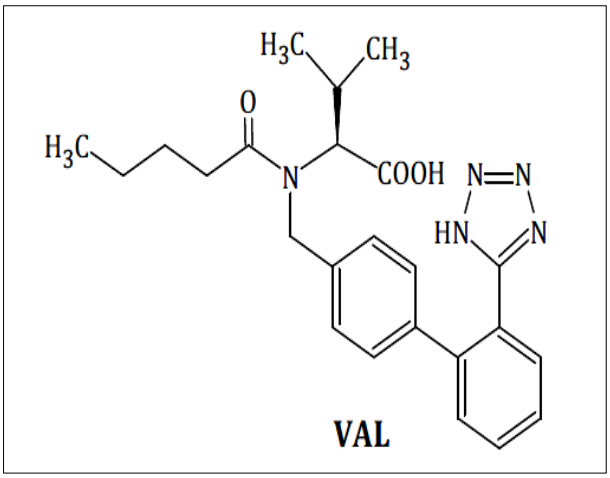
S.No	Drug	Valsartan		Hydrochlorothiazide	
		Proposed method	Reported method[35]	Proposed method	Reported method[35]
1	mean ± SD	100.163±1.464	100.38±0.784	100.346±0.599	100.69±0.909
2	N	3	3	3	3
3	RSD	1.462	0.782	0.599	0.909
4	V	2.143	0.614	0.358	0.826
5	SE	0.488	0.261	0.199	0.303
6	Student t-test	0.226 (2.78)*		0.547 (2.78)*	
7	F-test	3.490 (19.00)*		2.302 (19.00)*	

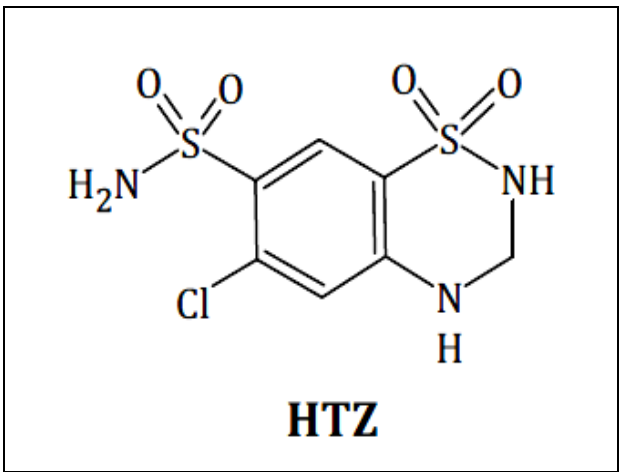
Table No.5: Robustness for the stability indicating chromatographic method of valsartan and hydrochlorothiazide mixture

S.No	Item		Flow rate (mL/min)		Acetonitrile content (%)		
			1.00	1.05	49.0%	50.0%	50.5%
1	1- Mixture of (32µg/mL VAL and 2- 5µg/mL HCT)	Resolution factor	21.01	22.35	22.46	21.01	22.56
2	3- Mixture of (64µg/mL VAL and 4- 10 µg/mL HCT)		22.89	22.14	22.55	22.89	22.27
3	5- Mixture of (96µg/mL VAL and 6- 15 µg/mL HCT)		20.52	21.57	20.85	20.52	20.89

Table No.6: Analysis of Valsartan and Hydrochlorothiazide mixture in Co-vasotec® tablet

S.No	parameter	Valsartan			Hydrochlorothiazide		
		Amount taken (µg/mL)	Amount found (µg/mL)	Recovery %	Amount taken (µg/mL)	Amount found (µg/mL)	Recovery %
1	Co-vasotec® 80/12.5mg tablet	32.0	32.672	102.100	5.0	5.061	101.220
		48.0	47.299	98.539	7.5	7.505	100.071
		64.0	64.022	100.034	10.0	10.022	100.222
		96.0	95.981	99.980	15.0	14.981	99.873
2	Mean ± SD			100.163±1.464			100.346±0.599
3	% RSD			1.462			0.597





(A) Valsartan **(B) Hydrochlorothiazide**

Figure No.1: Chemical structures of Valsartan and Hydrochlorothiazide

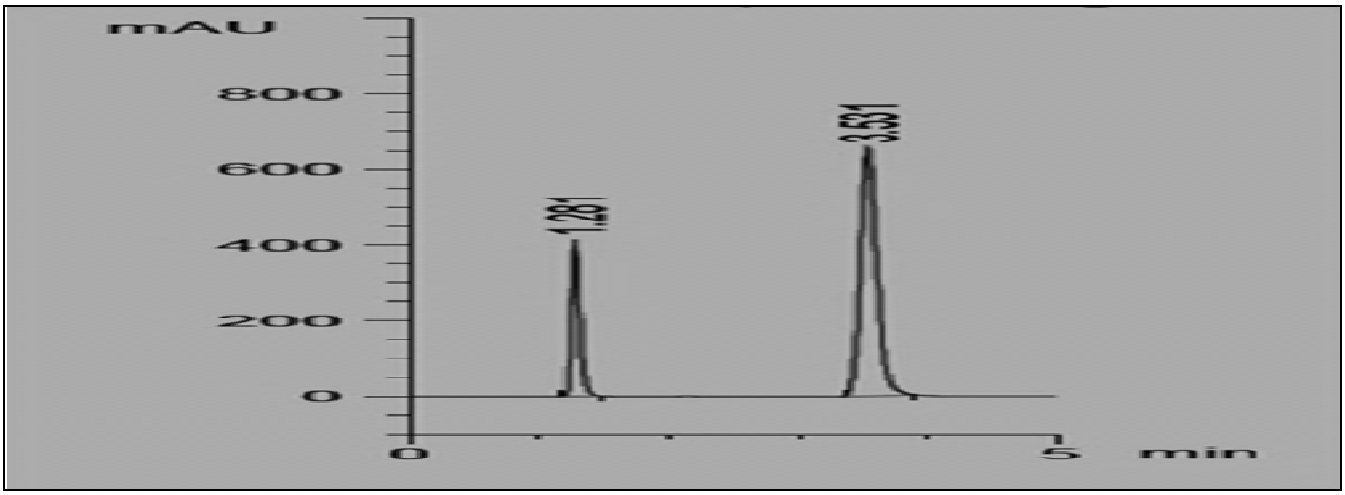


Figure No.2: Chromatogram of standard solution of 80 µg/mL valsartan and 12.5 µg/mL of hydrochlorothiazide

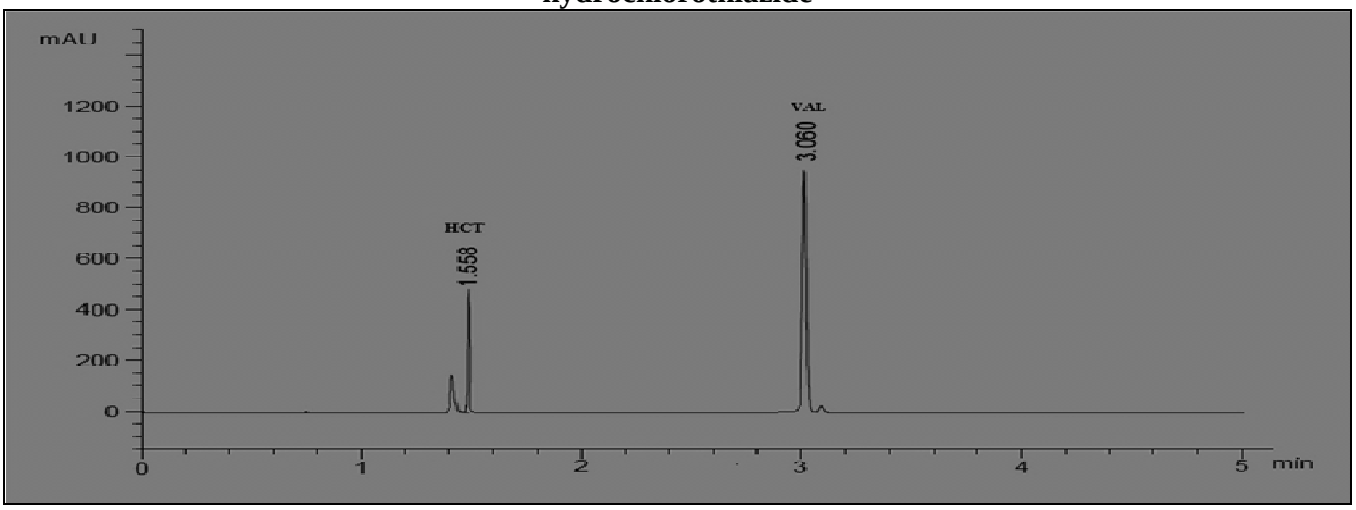
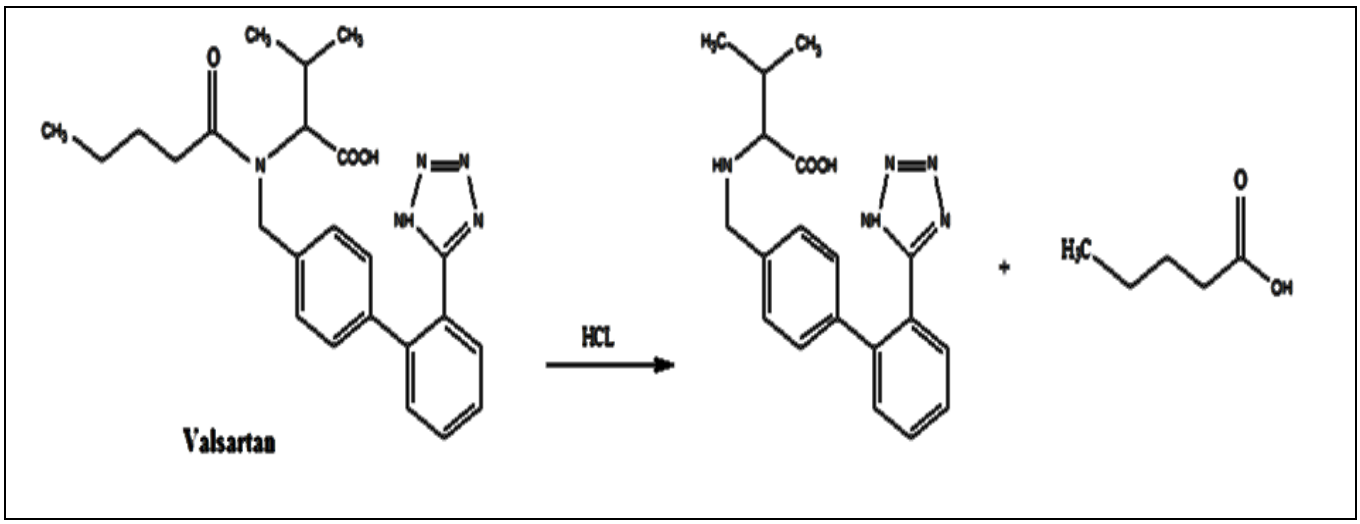
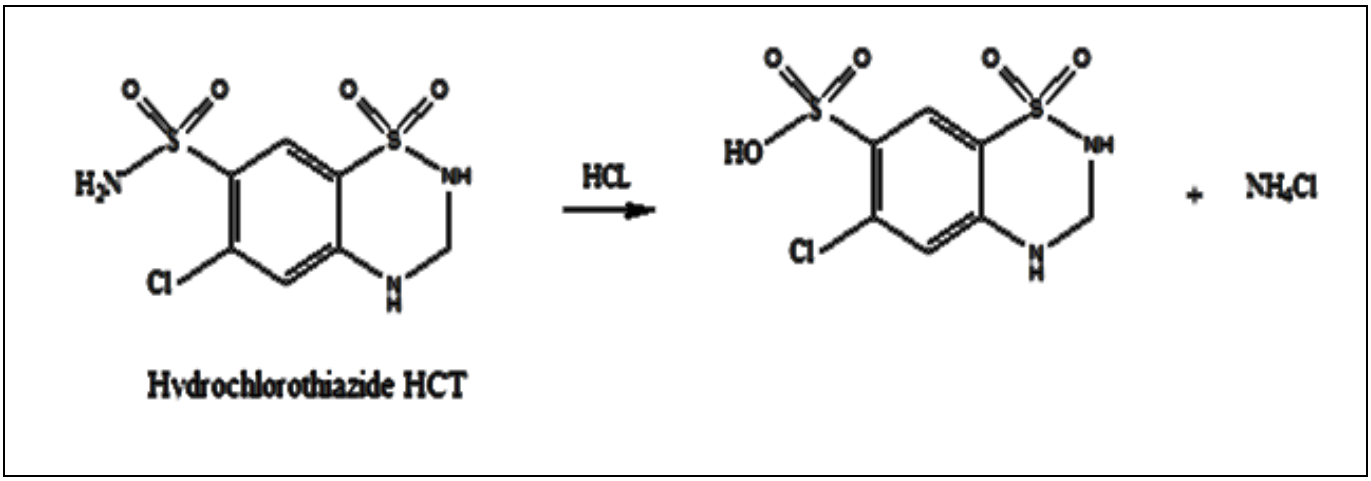


Figure No.3: Chromatogram of acidic degradation of valsartan and hydrochlorothiazide





Scheme No.1: Proposed reaction pathway for the reaction of VAL and HCT with HCL

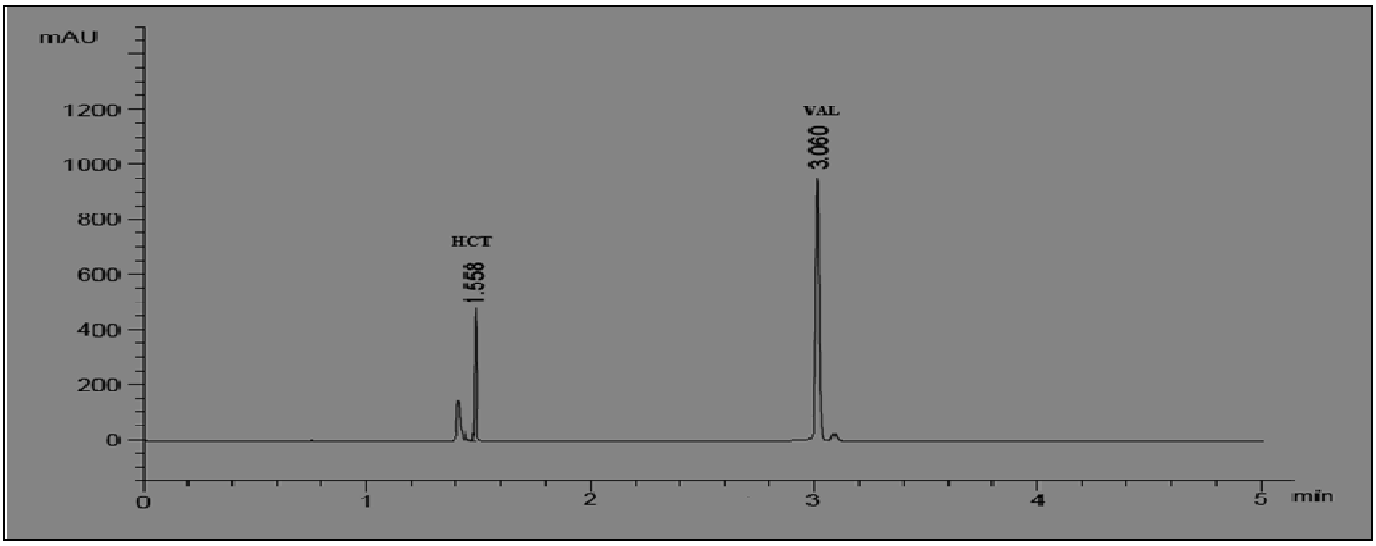
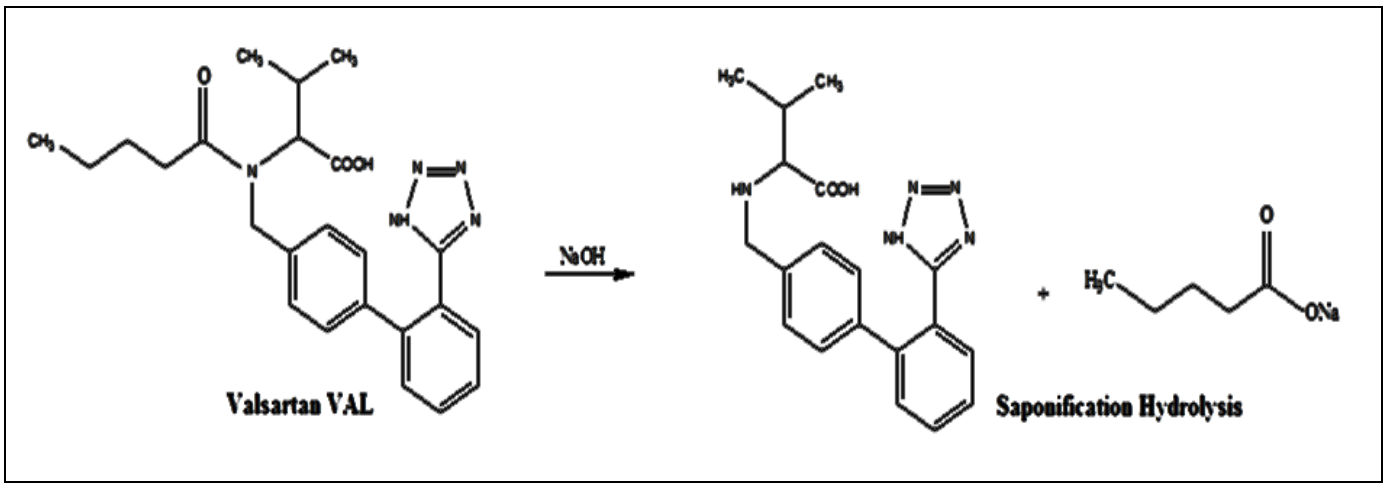
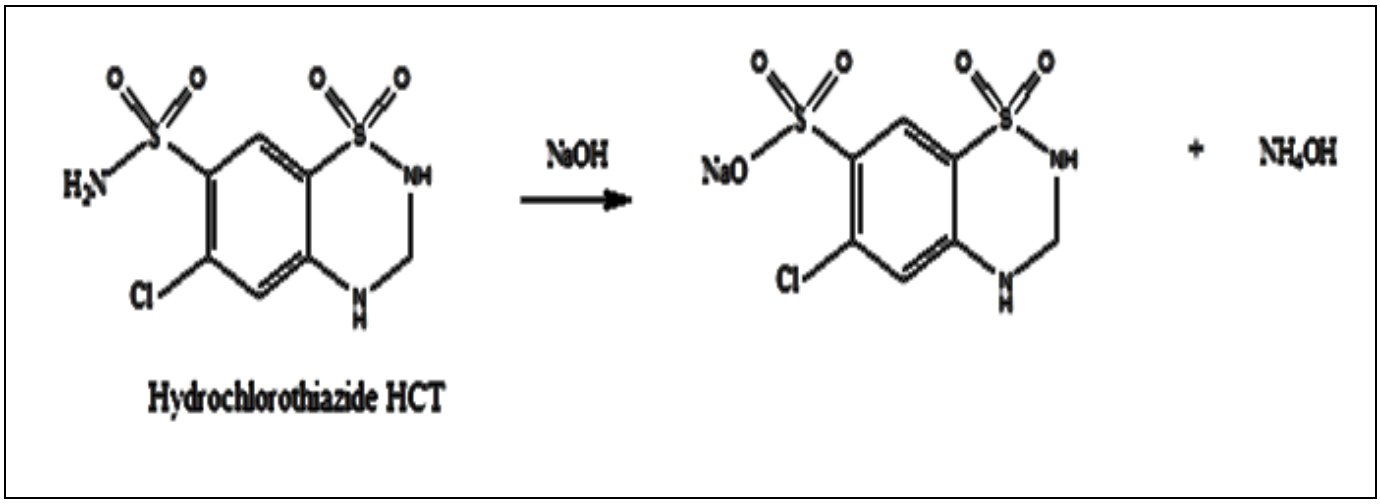


Figure No.4: Chromatogram of alkaline degradation of valsartan and hydrochlorothiazide





Scheme No.2: Proposed reaction pathway for the reaction of VAL and HCT with 0.1 NaOH Oxidative degradation

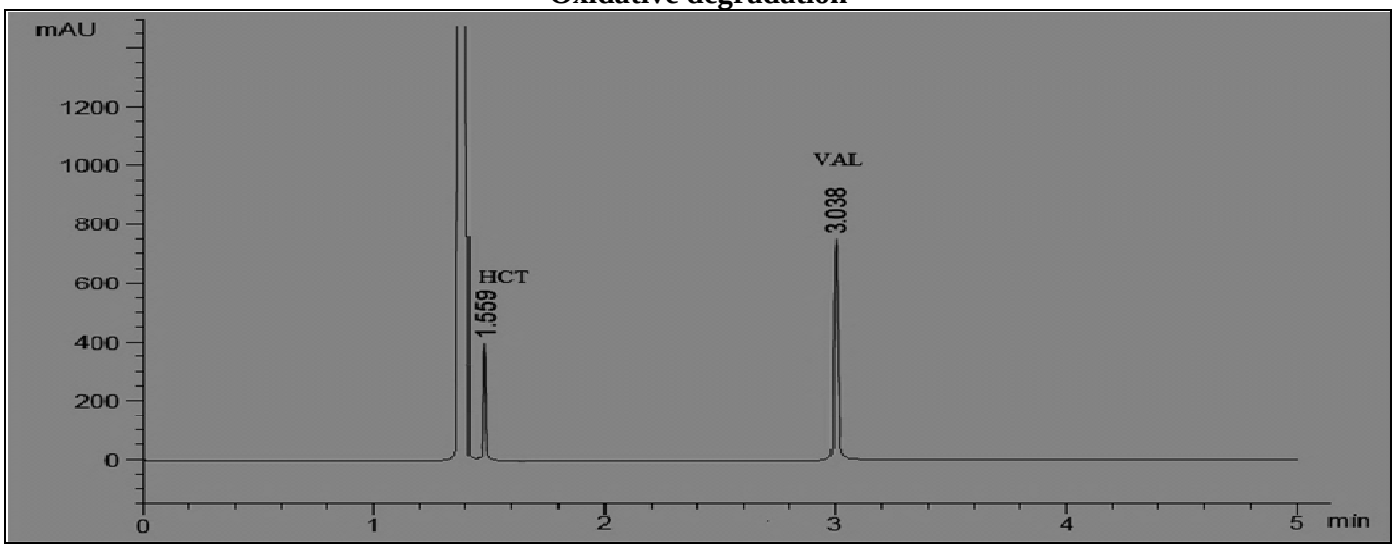
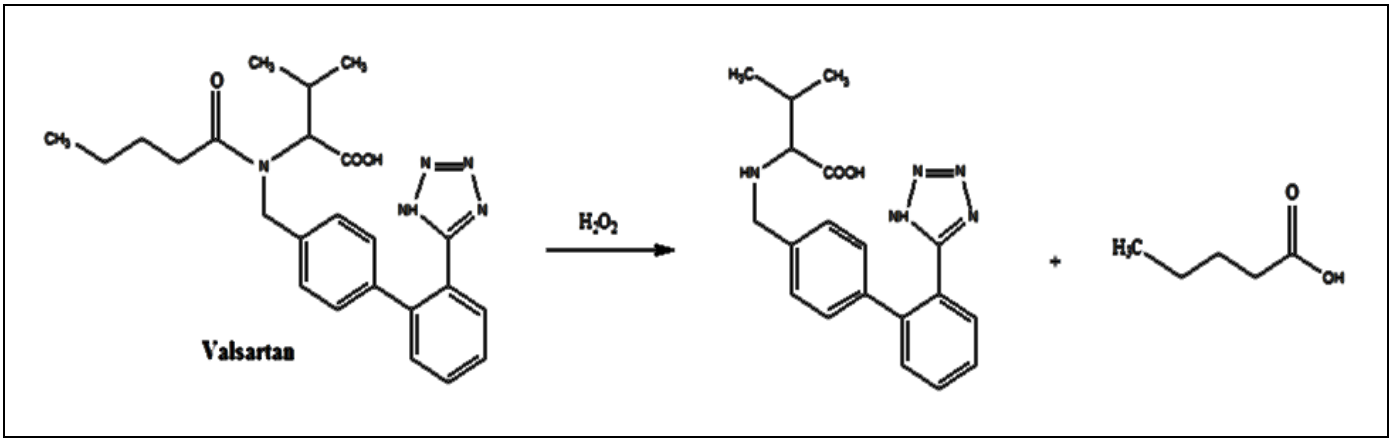
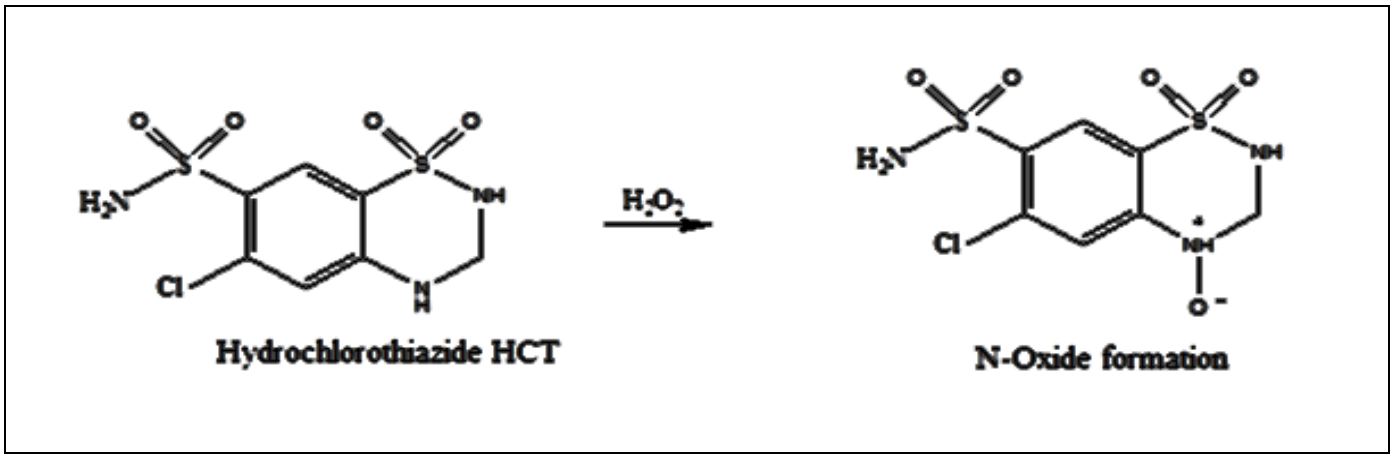


Figure No.5: Chromatogram of oxidative degradation of valsartan and hydrochlorothiazide





Scheme No.3: Proposed reaction pathway for the reaction of VAL and HCT with 30 % H_2O_2

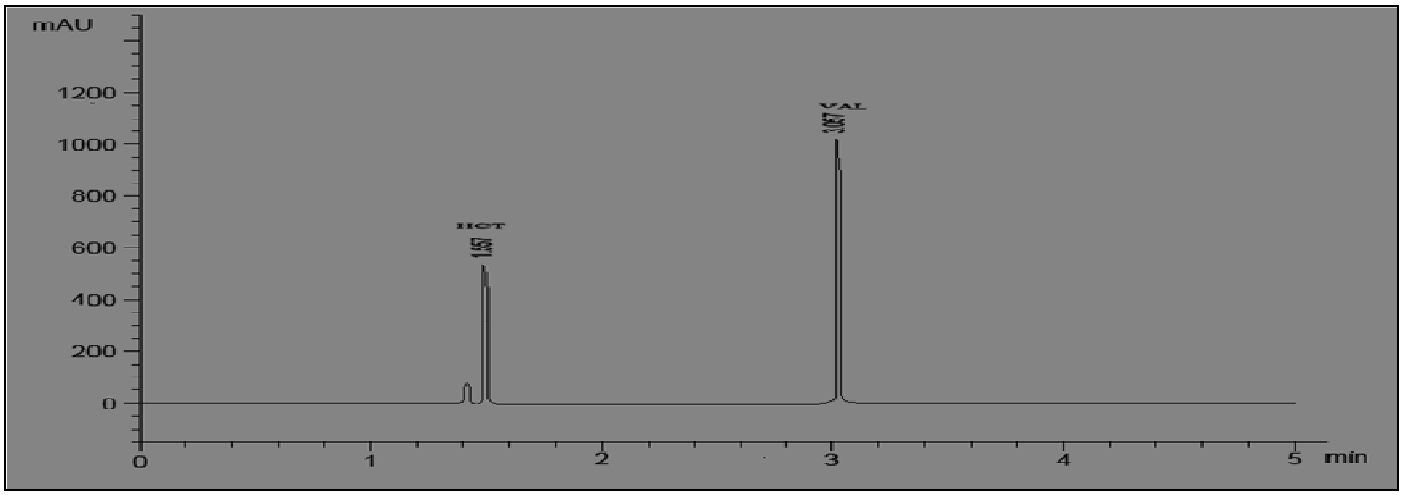


Figure No.6: Chromatogram of thermal degradation of valsartan and hydrochlorothiazide

Photolytic degradation

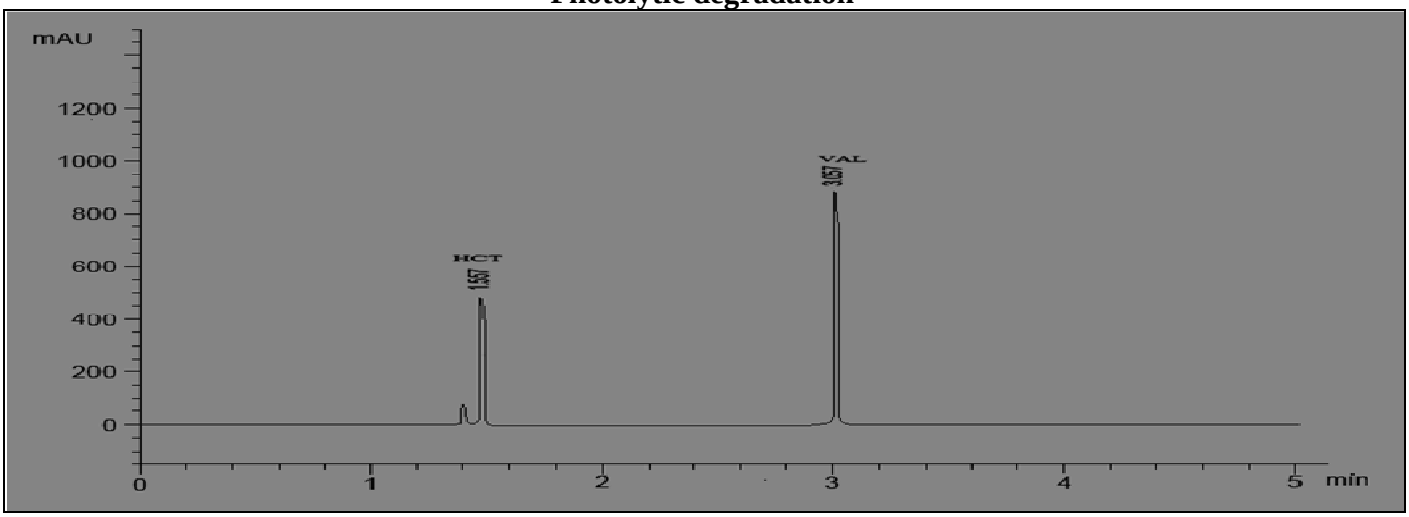


Figure No.7: Chromatogram of photo degradation of valsartan and hydrochlorothiazide

CONCLUSION

The present study represents the stability-indicating HPLC method for determination of VAL and HCT in their commercially available tablets. The proposed method showed acceptable accuracy, precision, selectivity, and concentration range. From the economical point of view, the method involved the native UV-absorbing property of VAL and HCT, rather than expensive derivatizing analytical reagents. Statistical analysis for the results proved that the method is suitable for the determination of VAL and HCT in bulk and its tablet without any interference from the degradation product and it is recommended for routine use in quality control industry laboratories.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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