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Development & Validation of a High Performance Liquid Chromatography Method for Simultaneous Determination of Irbesartan and Its Related Impurities in Pharmaceutical Tablets

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ABSTRACT

A novel isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for the determination of purity of Irbesartan drug substance in bulk samples and its pharmaceutical dosage forms in the presence of its impurities. This method is capable of separating related impurities along with Irbesartan. This method can be also be used for the estimation of assay of Irbesartan in drug substance as well as in single tablet formulation. Two impurities were detected in drug sample by HPLC analysis. The chromatographic conditions were optimized using an impurity-spiked solution. MS and IR method was used for the identification of impurities. The structure of the impurities were confirmed as 2-Cyano-4'-bromomethyl biphenyl and 2-n-butyl-1, 3-diazaspiro [4, 4]-non-1-ene-4-one. The method was subsequently validated for the determination of Irbesartan and its related compounds, as per ICH guidelines, for accuracy, precision, linearity and range, selectivity, limit of detection, limit of quantification and robustness. The LOD for Irbesartan, Impurity 1 and Impurity 2 was found to be 18.51µg/ml or ppm, 16.033µg/ml or ppm and 16.069µg/ml or ppm respectively while LOQ was found to be 56.098µg/ml or ppm, 48.587µg/ml or ppm and 48.69µg/ml or ppm respectively.

Keywords: Irbesartan, Impurity, HPLC, Structural elucidation, MS, Validation.

INTRODUCTION

Irbesartan is a nonpeptide tetrazole derivative, which is a potent, orally active, selective angiotensin II receptor (type AT1) antagonist. [1] Its main use is in hypertension (high blood pressure), diabetic nephropathy (kidney damage due to diabetes) and congestive heart failure. [2-3] Irbesartan, IUPAC name is 2-butyl-3-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl) phenyl] phenyl}methyl)-1,3-diazaspiro[4.4]non-1-en-4-one and molecular formula $C_{25}H_{28}N_6O$ (Fig. 1). EP and USP describe HPLC method for Irbesartan and its related impurities. [4-5] Spectroscopic methods are also reported for characterization of trace level impurities of Irbesartan. [6] GC-MS method to analyze genotoxic impurities is reported. [7] RP-HPLC method for quantification of impurity in Irbesartan is reported [8] but the impurities discussed in the present paper are not published, to the best of our knowledge.

Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients (API's) or develop during formulation or upon aging of API and tablet / suspension formulations.

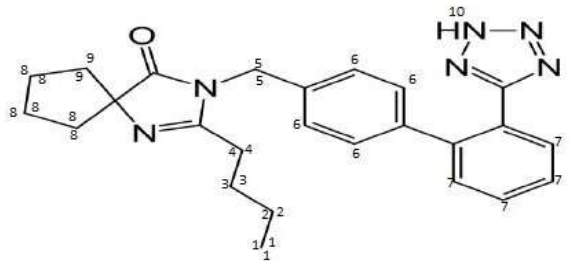

Fig. 1: Irbesartan

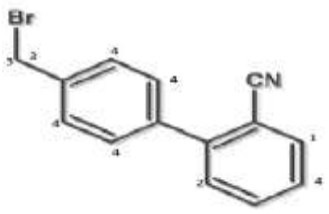

Fig. 2: Impurity1

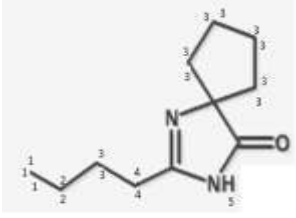

Fig. 3: Impurity2

Many potential impurities arise during the synthesis of API. The amount of these impurities present in drug substance (API / formulation) will determine the safety of drug product. [9] Therefore identification, quantification, qualification and

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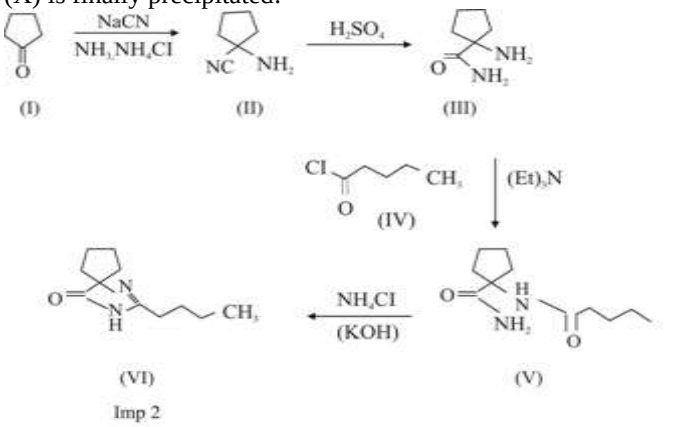
control of impurities are now crucial part of drug development. Chromatographic impurity profiles are most often developed using reversed-phase high-performance liquid chromatography (RP-HPLC). The chromatographic impurity profile should allow detecting and separating all (un)identified impurities in each new active compound.

Different methods of synthesis of Irbesartan are reported in the literature [10-16]. In present work Irbesartan synthesized from one of the route [10-11] was analysed by HPLC method.

The reaction of cyclopentanone (I) with sodium cyanide, NH_3 and NH_4Cl in hot methanol/water gives 1-aminocyclopentanecarbonitrile (II), which is partially hydrolyzed with concentrated H_2SO_4 to the corresponding amide (III). The acylation of (III) with pentanoyl chloride (IV) by means of triethylamine in THF yields 1-(pentanamido)cyclopentane-1-carboxamide (V), which, without isolation, is cyclized by means of KOH in refluxing methanol/water to afford compound (VI). Bromination of 4'-(methyl)biphenyl-2-carbonitrile (VII) with NBS gives compound (VIII). The condensation of compound (VI) with compound (VIII) by means of NaH in DMF gives intermediate compound (IX) which on cyclization with tributyltin azide or sodium azide gives Irbesartan (X). [10] Compound (IX) may also be treated with sodium azide and piperazine or its acid salt in a suitable organic solvent and resulting Irbesartan (X) obtained as its alkaline salt in aqueous solution. On neutralization with an acid Irbesartan (X) is finally precipitated. [11]



Synthesis of Imp 2

Synthesis of Imp1

Two impurities were detected in the drug formulation obtained by this process. Both have not been reported, by HPLC method, to be present in the dosage previously. Present paper describes the characterization of both the impurities present in Irbesartan drug formulation.

Thus, the aim of this study was to develop a liquid chromatograph that can simultaneously analyze Irbesartan and its two impurities, I and II. The method was validated in terms of precision, accuracy, linearity and range, selectivity, LOD, LOQ and robustness. The method utilizes a C_{18} column as stationary phase with photo diode array detector at 260 nm.

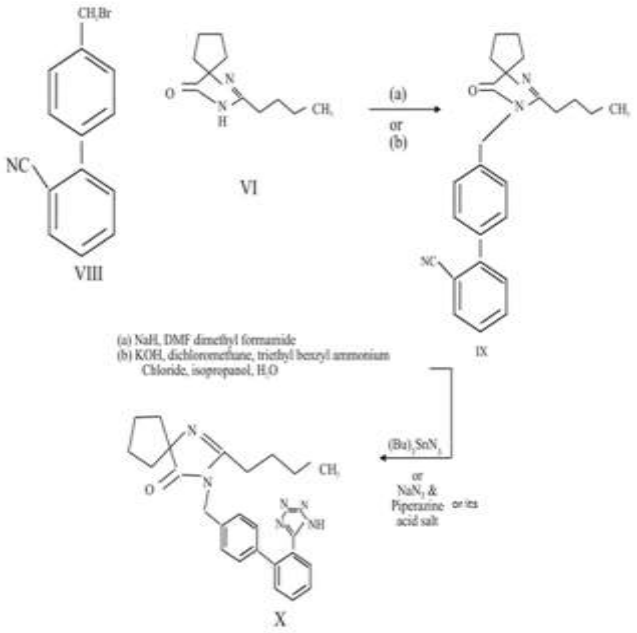


Fig. 4: Synthetic route for Irbesartan

I) Cyclopentanone (II) 1-aminocyclopentane carbonitrile (III) 1-aminocyclopentanamide (IV) pentanoyl chloride (V) 1-(pentanamido) cyclopentane-1-carboxamide (VI) Imp2 (VII) 4'-(methyl) biphenyl-2-carbonitrile (VIII) Imp 1 (IX) 4'-(2-butyl-4-oxo-1,3-diaza-spiro[4,4]non-1-en-3-ylmethyl)-biphenyl-2-carbonitrile (X) Irbesartan

EXPERIMENTAL

Material and Reagents

Irbesartan API sample was kindly provided by Vivan Life Science and pharmaceutical dosages were obtained from the market. HPLC grade methanol, acetonitrile, KH_2PO_4 H_3PO_4 and H_2O were purchased from Merck India Ltd. KBr (FTIR) grade was purchased from Merck KGaA, Germany. Impurity I and Impurity II were obtained from market to be used as standards. Excipients Mg-stearate, microcrystalline cellulose, Lactose monohydrate, Croscarmellose Na, Pregel starch was provided by Lubrizol Advanced Materials India (Life Science Polymers).

Instrumentation

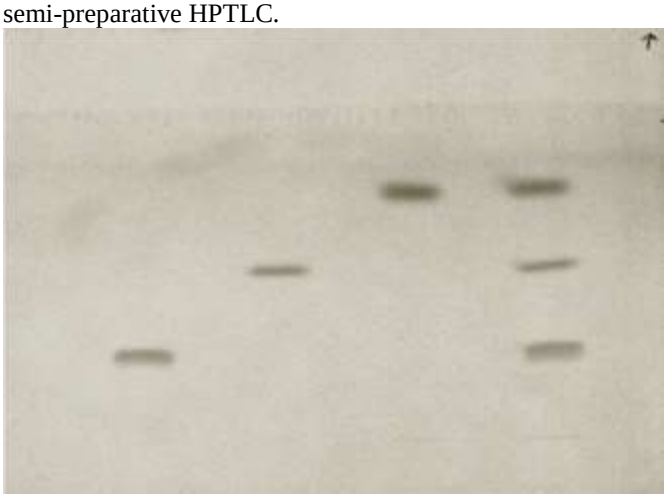
HPLC

HPLC analysis was performed using Schimadzu UFLC Prominence system. The LC solution software was employed for data processing and acquisition. LC-20 AD pump, DGU-20 A_3 degasser, CTO-20 AC column, SIL-20 AC HT autosampler, SPD – M20A photodiode array detector were during analysis. Different columns and mobile phases were tested. Finally, the method was validated with Phenomenex Luna C_{18} column with dimensions: Length: 250 mm, Diameter: 4.6 mm, Particle size: 5 micron and Pore size: 100 Armstrong. Isocratic elution technique was used. The mobile phase consisted of methanol: acetonitrile: buffer A (40: 30: 30) being buffer A: 0.005 M KH_2PO_4 with pH adjusted to 4.7 with orthophoshporic acid. The oven temperature was 25^{o}C and flow rate maintained at 0.5 ml / min. The UV detection was made at 260 nm.

Semi-preparative HPTLC

The impurities were isolated from the dosage formulation of Irbesartan using CAMAG Linomat 5 "Linomat5_08022"S/N 08022 (1:00:12) at dosage speed 150nl/s. The application volume was 200µL. CAMAG TLC Scanner "Scanner_170422" S/N 170422 (2:01:02) was used for

detection. The image was captured at 254 nm using CAMAG Visualizer: 150503 (Visualizer _150503). The mobile phase consisted of Toluene: Chloroform: Ethyl alcohol (4:4:1). The sample solution of 100 mg/ml was prepared in methanol for semi-preparative HPTLC.



a b c d

Fig. 5: HPTLC Image of (a) Irbesartan std,0.1µg/µL (b) Impurity2 std, 0.2µg/µL (c) Impurity1 std, 0.1µg/µL and (d) spiked

Mass spectrometry

The mass spectra of pure compound as well both the impurities was recorded using Varian Inc USA make spectrometer of model 410 Prostar Binary LC with 500 MS IT PDA detectors. The specification of instrument is as follows:

1. Direct infusion mass with ESI and APCI negative and positive mode ionization, mass ranging from 50 to 2000 m/e.
2. LCMS/MS and MS^n ion trap.
3. HPLC with PDA detector.
4. HPLC PDA detector – mass spectrometer.

IR spectroscopy

The IR spectra of Impurity I and Impurity II was recorded in the solid state KBr powder dispersion using an IR Prestige-21 Schimadzu spectrometer equipped with software IR probe.

NMR spectroscopy

The NMR spectra of both the impurities and the pure compound was recorded using Varian make spectrometer of 400 MHz having operating system unix and equipped with software vnmrj.

Sample preparation

Standard stock solution

In case of HPLC the standard stock solution of Irbesartan API, Impurity I and Impurity II was prepared by dissolving 25 mg of each in 5.0 ml of methanol (5000 ppm or µg /ml). 2.5 ml of each Irbesartan API, Impurity I and Impurity II was diluted to 25 ml in standard flask with methanol to give 500 ppm or µg /ml solution of each. Internal standard used was Losartan and its stock solution was prepared by dissolving 10 mg in 10 ml of methanol (100 ppm or µg /ml).

Sample solution

Twenty tablets from dosage form of Irbesartan were weighed and finely powdered with a mortar and pestle. A quantity of the powder equivalent to150 mg of Irbesartan was transferred into a 250 ml volumetric flask and methanol was added. The solution was sonicated for ten minutes and then the solution was completed to volume with the same solvent. This solution was filtered through a 0.2 µm nylon filter (Whatman, Dassel, Germany). 2.0 ml of the filtered solution along with 1.0 ml of internal standard was diluted to 10.0 ml with the solvent methanol. An aliquot of this solution was used for analysis.

Method Validation

The proposed method was validated according to the ICH guidelines [17] for its specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy, robustness and system suitability for Irbesartan and its impurities. [18] Assay for Irbesartan in pharmaceutical dosage formulation was also determined.

Specificity

The specificity of the developed method was examined for the presence of possible interference from excipients or sample matrix by overlaying chromatograms of API spiked with impurities, blank and drug products.

Linearity

Linearity was examined for the API of Irbesartan as well as Impurity I and Impurity II. 2.5 ml of each API, Impurity I and Impurity II of 5000 ppm or µg /ml were taken in a 25 ml standard flask and diluted with methanol to give a mixture stock solution which is 500 ppm or µg /ml with respect to API, Impurity I and Impurity II. For linearity studies fourteen concentrations from 0.01 ppm or µg /ml to 500 ppm or µg /ml were prepared with the help of mixture stock solution adding 1 ml of internal standard of 100 ppm or µg /ml to each of the different concentration and analysed.

Limit of detection (LOD) and Limit of quantification (LOQ): The LOD and LOQ for Irbesartan and its impurities were calculated based on the standard deviation of the response and the slope.

$DL = 3.3\,\sigma / S \qquad QL = 10\,\sigma / S$

σ - Standard deviation of the response signal

S – Slope of the calibration curve

Precision and accuracy

Repeatability (Intraday precision) was examined by three fold analyses of preparations of 150 ppm or µg /ml mixture of Irbesartan, Impurity I and Impurity II for three times in one day. Between days variation (Intermediate precision or Interday precision)) was examined on three consecutive days as per laboratory convenience. The % RSD on the peak areas was evaluated. Accuracy of the proposed method was determined by the standard addition method on the pharmaceutical dosage form to which known amounts of Irbesartan, Impurity I and Impurity II standards have been added at different concentrations .The determination was carried out at three level 80 %, 100% and 120%. The determination was carried out using three replicates at each concentration level. The accuracy was determined as percent recovery of amount of analyte added to the sample.

Robustness

To evaluate the robustness of the method, experimental factors that might cause variability in the method responses were examined. Usually the analytical parameters varied are composition and / or pH of mobile phase, column temperature and flow rate. But as per facilities available and convenience of the laboratory only two factors (column temperature, flow rate of mobile phase) were investigated. Three replicate analysis were carried out at each of three different column temperatures (20°C, 25°C, 30°C) and at three different flow rate of mobile phase (0.4 ml / min, 0.5 ml / min, 0.6 ml / min).

Table 1: NMR assignment of Irbesartan, Impurity1 and Impurity2

Position[a]	1H	δppm	Position[b]	1H	δppm	Position[c]	1H	δppm
1	3H	0.8	1	1H	8.0	1	3H	0.8
2	2H	1.25	2	1H	7.8	2	2H	1.25
3	2H	1.45	3	2H	4.8	3	11H	1.65-2.0
4	2H	2.2	4	6H	7.6	4	2H	2.8
5	2H	4.6				5	1H	13-14 hump
6	4H	7.0						
7	4H	7.6-7.8						
8	6H	1.9						
9	2H	1.7						
10	-		Due to high electronegativity of N- atoms the signal is highly deshielded					

[a]Refer the structural formula in Fig. 1; [b]Refer the structural formula in Fig. 2; [c]Refer the structural formula in Fig. 3

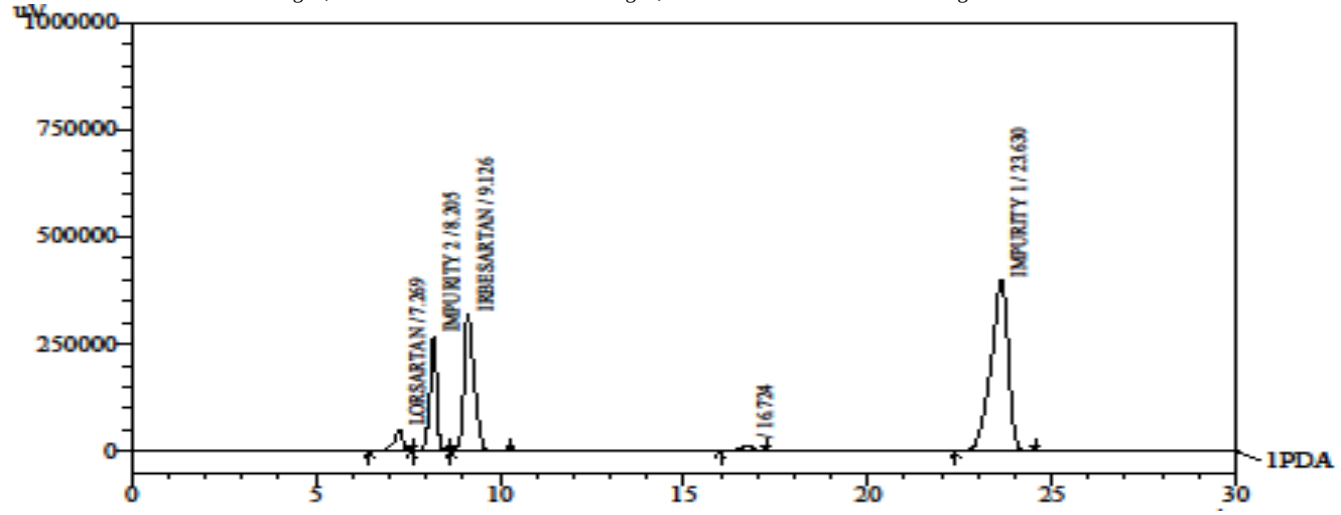


Fig. 6: HPLC chromatogram of Irbesartan, Impurity1 and Impurity2 and internal standard Losartan

Forced degradation study

Forced degradation or stress testing involves exposure of drug substance to heat, heat and humidity, light or range of pH values. In this case hydrolytic study under acidic and basic condition was carried out as it involves catalyzation of ionisable functional groups present in the molecule. HCl and NaOH were employed for generating acidic and basic stress samples respectively. A quantity of the powder equivalent to 25 mg of Irbesartan was transferred into each of the two 25 ml volumetric flask and methanol was added. The solution was sonicated for ten minutes, and then 1.25 ml of 1 N HCl and 1.25 ml of 1 N NaOH was added to each of the two standard flask. The solution was diluted to volume with methanol. Both the solutions were kept in dark for 24 hours. 2.0 ml of the solution was diluted to 10.0 ml with the solvent methanol. An aliquot of this solution was used for analysis.

RESULTS AND DISCUSSION

Method development

The method was developed as described above.

Detection of impurity by HPLC and LC/MS

The Irbesartan samples prepared by known synthetic route [10] (Fig. 4) were analysed by using HPLC method as described above. The analysis revealed the presence of two impurities. The impurities were marked as Impurity1 (RT 23.63 min) and Impurity2 (RT 8.205 min) respectively. (Fig 6). The retention time of both the impurities matched with API sample of Irbesartan containing internal standard Losartan and spiked Impurities 1 and 2.

To further investigate these impurities, LC/MS compatible method described above was developed. Mass spectral data showed molecular protonated parent ion peak at m/z 429 for Irbesartan, parent ion peak at m/z 192 for Impurity 1 (due to dissociation of Br^-), molecular protonated parent ion peak at m/z 195 for Impurity2. On the basis of spectral data, the Impurity1 having parent ion peak at m/z 192 is identified as 2-Cyano-4'-bromomethyl biphenyl while Impurity2 having protonated molecular ion peak at m /z 195 is identified as 2-butyl-1,3-diazaspiro[4,4]non-1-en-4-one.

Table 2: Regression Characteristics of the proposed HPLC method

	IRB	IMP 1	IMP 2
Range	25 - 250 µg/ml	5 - 250 µg/ml	0.5 - 210 µg/ml
Mean R^2 value	0.996	0.998	0.998
Slope m	0.051	0.111	0.029
Intercept c	-0.012	-0.111	-0.037

Criteria : Linear when corr. coefficient > 0.99

Table 3: LOD & LOQ

	IRB	IMP 1	IMP 2
Range	0.5 - 250µg/ml	0.1 - 250 µg/ml	0.5 - 210 µg/ml
LOD	18.510 µg/ml	16.033 µg/ml	16.069 µg/ml
LOQ	56.098 µg/ml	48.587 µg/ml	48.690 µg/ml

Table 4: Precision

	* Intra day precision (Repeatability) RSD (n=9)	** Inter day precision RSD(n=9)
IRB	0.5054	0.9327
IMP 1	0.9072	1.7848
IMP 2	0.6322	1.3390
* Criterion (Drug) system RSD < 1.5 %		** Criterion (Drug) : RSD < 2.5%
* Criterion (Drug) method RSD < 2.0 %		** Criterion (IMP) : RSD < 10.0%
*Criterion (IMP)system & method RSD (100% - 200%) < 5%		

Table 5: Accuracy

	Percentage Recovery		
	Level 1	Level 2	Level 3
IRB	101.29756 ± 0.0175	101.8413 ± 0.0177	103.4753 ± 0.0450
IMP 1	101.6764 ± 0.0269	99.7114 ± 0.0833	103.4763 ± 0.3918
IMP 2	101.9669 ± 0.0104	103.4106 ± 0.0108	104.6058 ± 0.1083

Criterion (Drug)for mean recovery: 98-102%
Criterion for (IMP) mean recovery at 100.0 and 200.0%: 90-110%

Table 6.1 Temperature

	30°C					25°C					20°C				
	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor
Irbesartan	8.559	7.385	42.264	5915.200	0.000	8.836	7.956	42.857	5833.353	1.461	9.106	7.332	43.267	5778.076	1.852
	8.581	7.418	42.137	5933.028	1.216	8.844	7.669	42.080	5941.065	1.590	9.086	7.358	44.367	5634.819	1.827
	8.587	7.384	42.297	5910.585	1.202	8.847	8.109	42.784	5843.306	1.458	9.122	7.348	44.445	5624.930	1.702
	8.576	7.395	42.233	5919.604	1.209	8.842	7.912	42.574	5872.575	1.503	9.105	7.346	44.026	5679.275	1.794
	% Recovery = 93.843					% Recovery = 100.393					% Recovery = 93.215				

Criterion : Number of theoretical plates (n) n > 3000

- Tailing Factor (T) 0.9 < T < 2.0

	30°C					25°C					20°C				
	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor
Impurity 1	21.697	11.755	11.811	21166.710	1.055	23.114	12.602	11.582	21585.220	1.051	23.694	11.622	10.647	23480.790	1.039
	21.702	11.749	11.784	21215.210	1.055	23.125	12.602	11.581	21587.080	1.051	24.523	8.406	8.989	27811.770	0.891
	21.711	11.695	11.786	21211.610	1.055	23.132	12.799	11.591	21568.460	1.050	24.572	7.050	7.686	32526.670	0.848
	21.703	11.733	11.794	21197.843	1.055	23.124	12.667	11.585	21580.253	1.051	24.263	9.026	9.107	27939.743	0.926
	% Recovery = 72.027					% Recovery = 77.763					% Recovery = 55.408				

	30°C					25°C					20°C				
	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor
Impurity 2	8.059	4.332	17.143	14583.210	0.000	8.178	4.545	16.948	14751.000	1.188	8.295	4.125	17.061	14653.300	1.180
	8.059	4.312	17.116	14606.220	1.265	8.184	4.399	16.713	14958.420	1.160	8.292	4.140	17.334	14422.520	1.190
	8.063	4.294	17.050	14662.760	1.269	8.185	4.629	16.953	14746.650	1.189	8.299	4.153	17.027	14682.560	1.185
	8.060	4.312	17.103	14617.397	0.845	8.182	4.524	16.871	14818.690	1.179	8.295	4.139	17.141	14586.127	1.185
	% Recovery = 97.695					% Recovery = 102.491					% Recovery 93.771				

Table 6.2 Flow Rate

	0.6 ml/min					0.5 ml/min					0.4 ml/min				
	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor
Irbesartan	7.386	7.608	44.568	5609.406	1.730	8.845	8.063	42.943	5821.671	1.578	11.183	7.672	42.564	5873.508	1.642
	7.395	7.295	44.575	5608.525	1.683	8.854	7.777	42.530	5878.204	1.806	11.067	7.424	41.434	6033.692	1.778
	7.390	7.532	44.538	5613.184	1.628	8.852	7.820	42.877	5830.632	1.770	11.037	7.469	40.940	6106.497	1.474
	7.390	7.478	44.560	5610.372	1.680	8.850	7.887	42.783	5843.502	1.718	11.096	7.522	41.646	6004.566	1.631
	% Recovery = 94.894					% Recovery = 100.078					% Recovery = 95.444				

Criterion : Number of theoretical plates (n) n > 3000

Tailing Factor (T) 0.9 < T < 2.0

	0.6 ml/min					0.5 ml/min					0.4 ml/min				
	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor
Impurity 1	19.250	12.107	11.806	21175.670	1.050	23.058	12.871	11.581	21587.080	1.051	28.799	12.145	11.653	21453.700	1.055
	19.252	11.612	11.786	21211.610	1.050	23.065	12.586	11.605	21542.440	1.051	28.816	11.693	11.634	21488.740	1.055
	19.249	11.988	11.797	21191.830	1.050	23.070	12.418	11.602	21548.010	1.052	28.811	11.734	11.662	21437.150	1.055
	19.250	11.903	11.796	21193.037	1.050	23.064	12.625	11.596	21559.177	1.051	28.809	11.857	11.650	21459.863	1.055
	% Recovery = 73.067					% Recovery = 77.500					% Recovery = 72.789				

	0.6 ml/min					0.5 ml/min					0.4 ml/min				
	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor	Ret. Time	Area	HETP	n	Tailing factor
Impurity 2	6.821	4.296	18.623	13424.260	1.193	8.175	4.570	16.953	14746.650	1.187	10.216	4.317	15.795	15827.790	1.179
	6.822	4.129	18.507	13508.400	1.191	8.179	4.376	17.031	14679.110	1.172	10.231	4.163	15.945	15678.900	1.184
	6.816	4.274	18.492	13519.360	1.193	8.178	4.404	17.200	14534.880	1.187	10.224	4.217	15.703	15920.520	1.182
	6.820	4.233	18.541	13484.007	1.192	8.177	4.450	17.061	14653.547	1.182	10.224	4.232	15.814	15809.070	1.182
	% Recovery = 95.895					% Recovery = 100.813					% Recovery = 95.873				

Structural confirmation by NMR and IR

The NMR and IR spectral data of Impurity1 and Impurity2 confirmed the structures of both the compounds.

The H^1 NMR spectral data of Irbesartan, Impurity 1 and Impurity 2 were recorded (Table 1).

The IR spectrum of Impurity1 shows peaks at 2221.13 cm^{-1} (CN stretching), 1594.23 cm^{-1}, 1561.44 cm^{-1} (C=C aromatic stretching) and 643.29 cm^{-1} (aromatic substitution). The IR spectrum of Impurity2 shows peaks at 3527.96 cm^{-1}(N-H stretching) 1643.42 cm^{-1} (C=O stretching) 1518.04 cm^{-1} (N-H bending) 1421.6 cm^{-1} (CN stretching) 1458.25 cm^{-1} (C-H bending in cyclopentane).

Specificity

The specificity of the developed method was determined by examining the presence of possible interference from excipients or sample matrix. The chromatogram overlay of the spiked mixture of impurities and APIs, the drug product and the blank showed that the proposed method is specific for both APIs and their related substances as there was no any interference at the retention time of Irbesartan and their

related impurities. Thus complete separation was noticed in presence of tablet placebo and the peaks were pure and excipients in the formulation did not interfere the analysis.

Table 7: Assay

	Area (Irb/IS) n=3	Amount of drug present mg	% Assay
Std	8.4599	150	
Wt of powder of tablet (403.1mg)	6.8756	152.3882	101.5921

Table 8: Degradation study

Conditions	% Assay of active substance	Retention time of drug	% Degradation
No stress treatment	101.592	9.287	Nil
Acid degradation (1N HCl)	99.235	10.023	0.977
Alkaline degradation (1N NaOH)	98.329	8.778	0.968

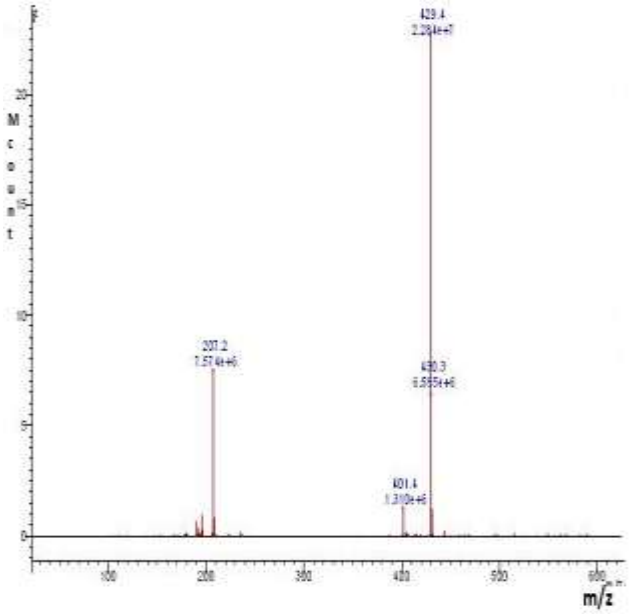


Fig. 7: Mass spectrum of Irbesartan

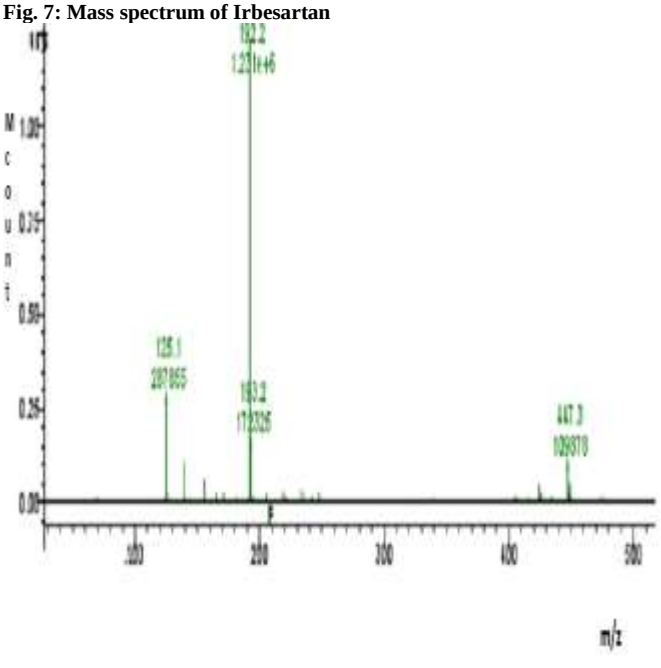


Fig. 8: Mass spectrum of Impurity1

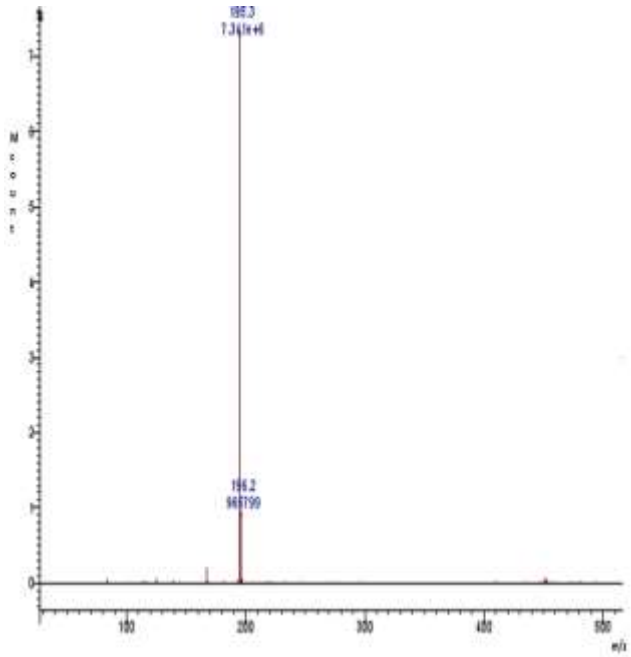


Fig. 9: Mass spectrum of Impurity 2

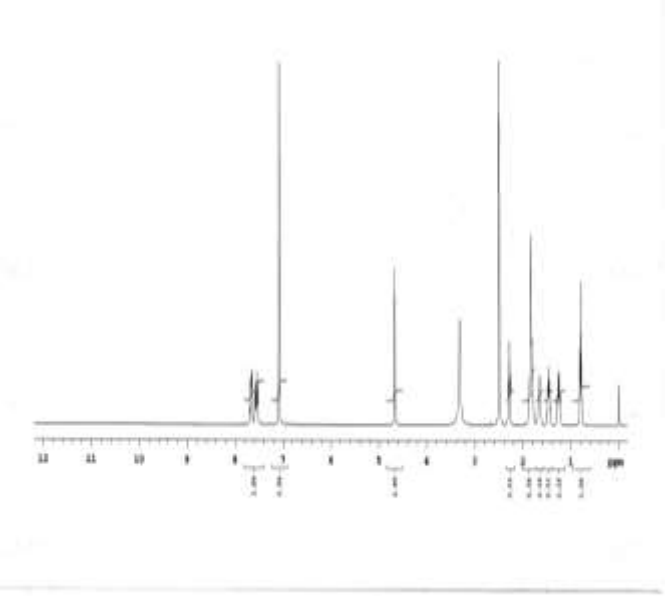


Fig. 10: ^{1}H NMR spectrum of Irbesartan

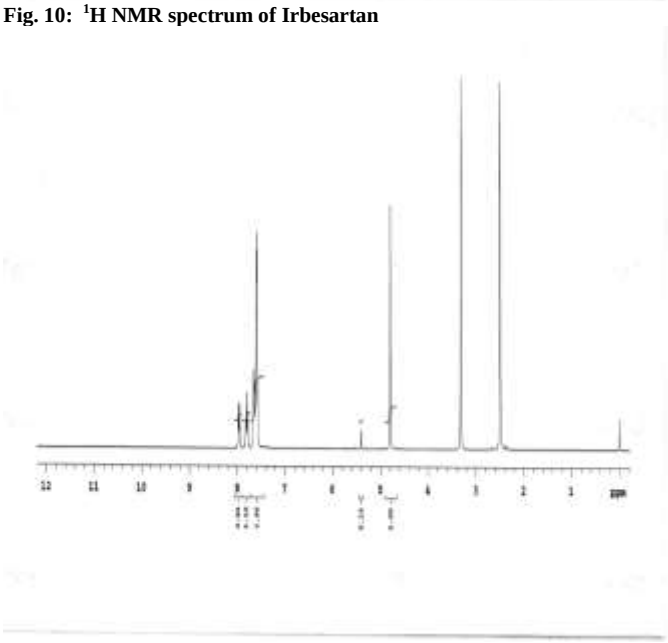


Fig. 11: ^{1}H NMR spectrum of Impurity1

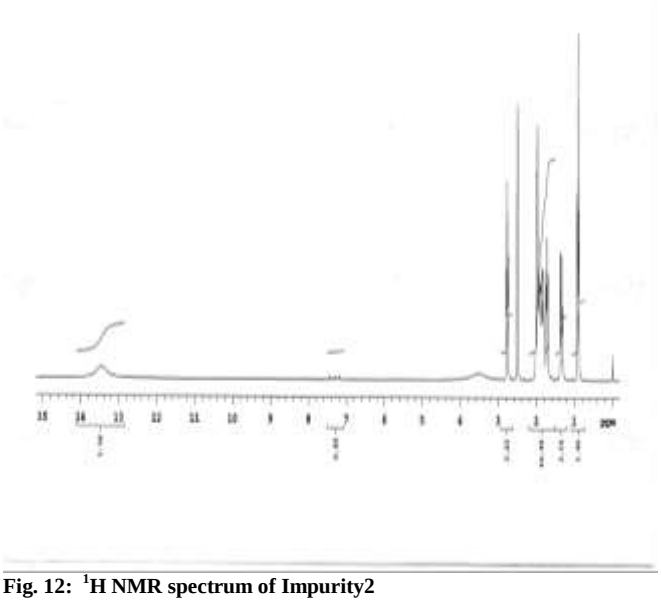


Fig. 12: ^{1}H NMR spectrum of Impurity2

Linearity

In the examined concentration range described above, linear responses were observed between the peak areas and the concentration of the analytes. The results indicate that the response is linear over the range of 25, 50, 90, 120, 150, 180, 210, 250µg/ml or ppm for Irbesartan. For Imp 1 linearity was observed over the range of 5, 10, 25, 50, 90, 120, 150, 180, 210, 250µg/ml or ppm and for Imp 2 linearity was observed over the range 0.5, 1.0, 5, 10, 25, 50, 90, 120, 150, 180, 210µg/ml or ppm. The coefficients of determination of the regression lines and the linear regression equations are shown in Table 2.

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ for Irbesartan and its related impurities were determined by injecting a series of dilutions of known concentrations of the analytes. It was found that for Irbesartan the LOD and LOQ was 18.51µg/ml or ppm and 56.098µg/ml or ppm respectively. For Imp 1, the LOD and LOQ were found to be 16.033µg/ml or ppm and 48.587µg/ml or ppm respectively. For Imp 2, the LOD and LOQ were 16.069µg/ml or ppm and 48.69µg/ml or ppm (Table 3).

Precision and accuracy

The precision of the method was evaluated as repeatability and intermediate precision. Repeatability was examined by three fold analyses of preparation of 150µg/ml or ppm of each of Irbesartan API, Imp 1 and Imp 2 in one day. The RSD on the peak areas of these determinations was not more than 1.0% for each. Intermediate precision was also determined for three consecutive days. The RSD on the peak areas of these determinations was not more than 2.0% for each suggesting that the proposed method is suitable for simultaneous analysis of Irbesartan and its related impurities. In addition, the intermediate precision suggests that the developed method gave repeatable results for three consecutive days. Accuracy of the method was determined as % recovery of a known added amount of analyte to the sample. The proposed method was found to give a mean percentage recovery of 102.2047 ± 0.026733 for Irbesartan, 101.6214 ± 0.16734 for Imp 1 and 103.3278 ± 0.04317 for Imp2 in the examined dosage form,as shown in Table 5. So the developed method gave satisfactory recoveries for Irbesartan, Imp 1 and Imp 2.

Robustness

The robustness of the HPLC method was checked by introducing intentional variation of the experimental factors as described above. The results, as shown in table 6, of the deliberate aforementioned changes in the parameters are in compliance with the conditions maintained for development of the method.

Application to real samples

The proposed method was applied for the determination of Irbesartan in commercial tablet formulation as described above. The content of Irbesartan in the tablet complies with the prescribed limit. The impurities are present below the restricted level specified under ICH guidelines (Table 7).

Stability indicating property

The chromatogram of acid degraded sample and alkaline degraded sample did not show any prominent additional peak. The chromatogram indicated that there was no appreciable loss in content of active component. The negligible peaks observed were from its blank or placebo in each of the specified condition. This indicates that the drug is not susceptible to acid or base hydrolysis degradation (Table 8).

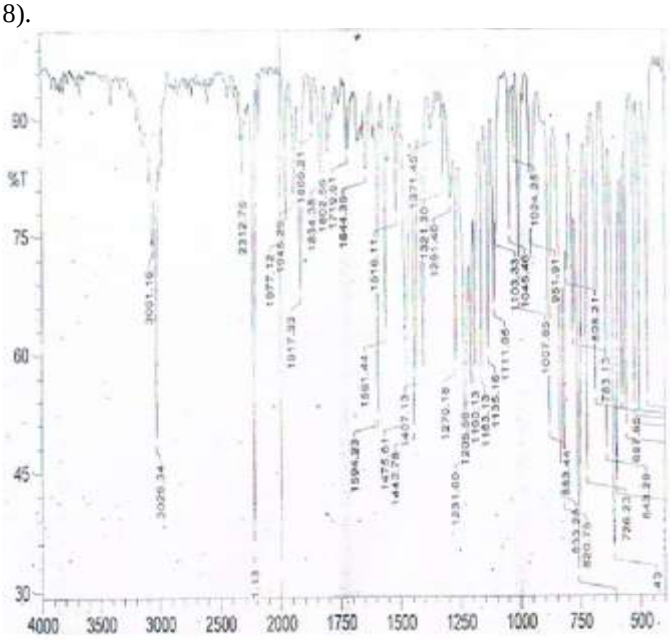


Fig. 13: IR spectra of Impurity1

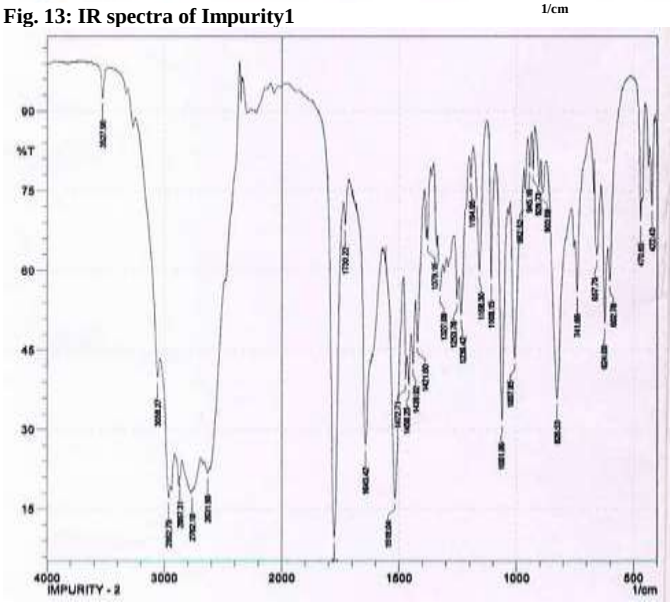
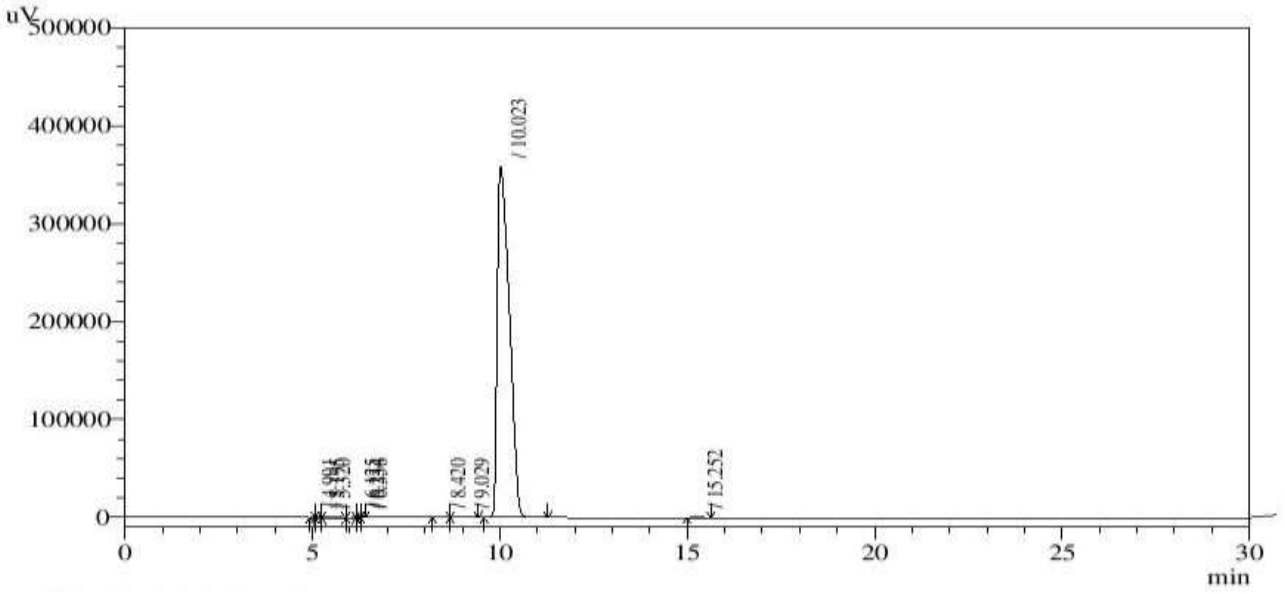


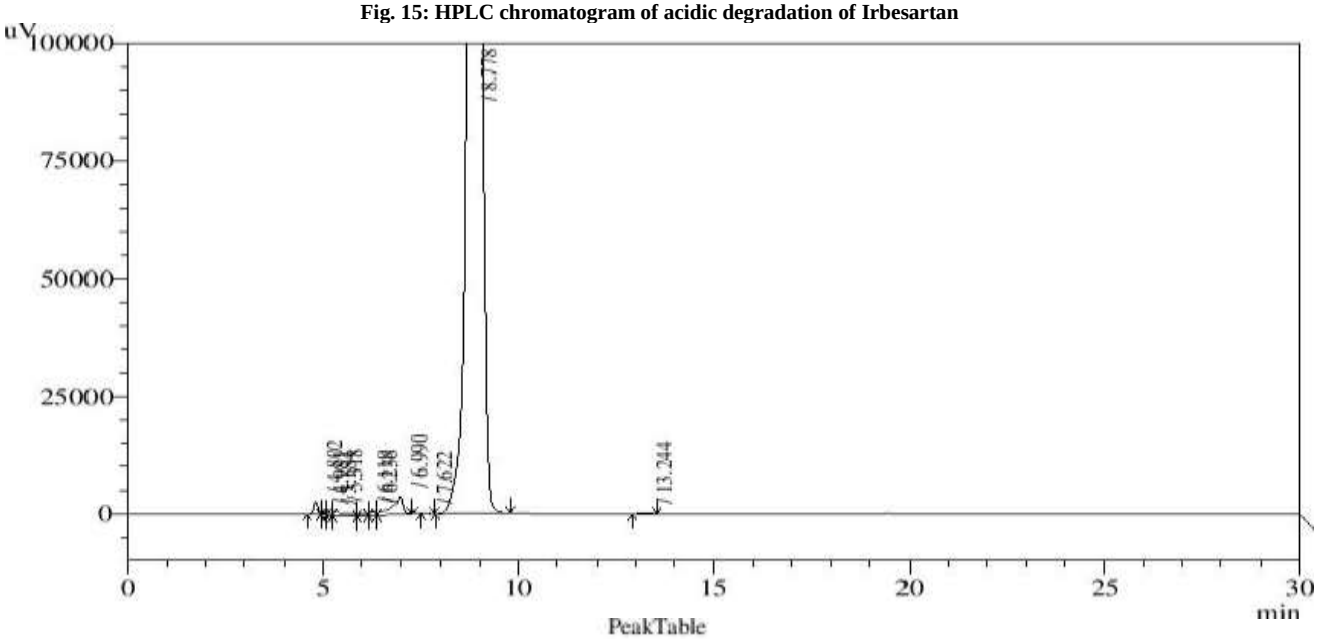
Fig. 14: IR spectra of Impurity2



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %	Tailing Factor
1	4.991	2391	362	0.030	0.100	0.000
2	5.195	5357	617	0.066	0.170	0.000
3	5.320	22949	1113	0.284	0.307	0.000
4	6.125	4168	439	0.052	0.121	0.000
5	6.234	2514	444	0.031	0.122	0.000
6	6.336	1123	243	0.014	0.067	0.000
7	8.420	4571	329	0.057	0.090	0.000
8	9.029	13729	855	0.170	0.235	0.988
9	10.023	8007429	358427	99.235	98.717	1.966
10	15.252	4921	258	0.061	0.071	1.176
Total		8069152	363085	100.000	100.000	

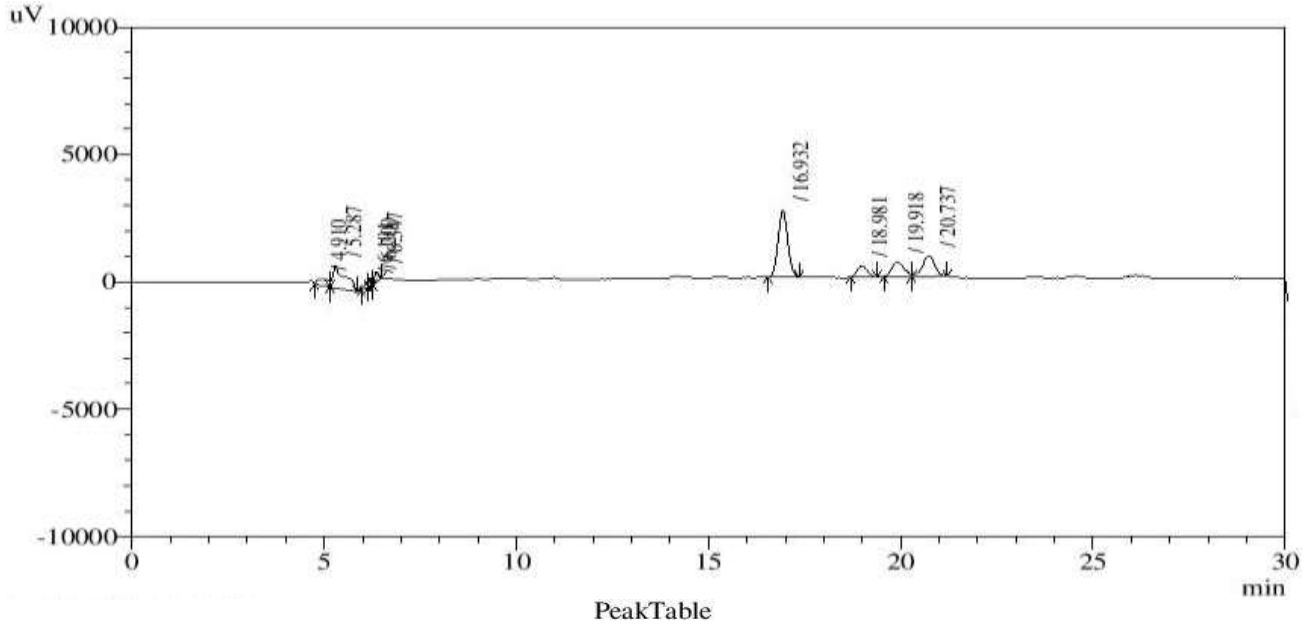
Fig. 15: HPLC chromatogram of acidic degradation of Irbesartan



PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %	Tailing Factor
1	4.802	21897	2693	0.255	0.587	0.000
2	4.981	2658	450	0.031	0.098	0.000
3	5.184	3930	496	0.046	0.108	0.000
4	5.318	18815	1063	0.219	0.232	0.000
5	6.119	7465	748	0.087	0.163	0.000
6	6.236	9303	838	0.108	0.183	0.000
7	6.990	73121	3863	0.853	0.842	0.000
8	7.622	1760	202	0.021	0.044	1.060
9	8.778	8431104	448510	98.329	97.697	1.350
10	13.244	4320	218	0.050	0.048	0.999
Total		8574373	459082	100.000	100.000	

Fig. 16: HPLC chromatogram of alkaline degradation of Irbesartan



Peak#	Ret. Time	Area	Height	Area %	Height %	Tailing Factor
1	4.910	5083	247	4.450	3.791	0.000
2	5.287	19749	868	17.291	13.301	0.000
3	6.101	1480	222	1.296	3.404	0.000
4	6.200	1217	327	1.066	5.004	0.000
5	6.347	2922	423	2.559	6.481	1.426
6	16.932	45659	2601	39.976	39.855	1.077
7	18.981	7908	440	6.924	6.742	1.157
8	19.918	12139	574	10.628	8.800	0.000
9	20.737	18059	824	15.811	12.623	0.000
Total		114217	6526	100.000	100.000	

Fig. 17: HPLC chromatogram of Blank

The developed reversed phase HPLC method is specific, linear, sensitive, precise and accurate for the separation and determination of Irbesartan and its impurities. The method can be applied for routine quality control of APIs and oral dosage forms.

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