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Research Article

Development and Validation of UPLC Method for Simultaneous Determination of Irbesartan, Hydrochlorothiazide, Amlodipine Besylate and Atorvastatin

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Abstract

A simple, accurate, precise, selective and sensitive UPLC method for simultaneous determination of four antihypertensive drugs Hydrochlorothiazide, Amlodipine, Irbesartan and Atorvastatin is developed. The good chromatographic separation was achieved by using a ACQUITY UPLC BEH C_{18} 2.1 x 150 mm, 1.7 µm column and a mobile phase consisting of 0.01 M potassium dihydrogen phosphate buffer : acetonitrile (70:30) (v/v) pH 5 while at a flow rate of 0.25 ml min^{-1}. Hydrochlorothiazide, Amlodipine, Irbesartan and Atorvastatin were detected at 254 nm and were eluted at 2.9, 3.2, 3.6 and 6.1 min respectively. The method was validated with respect to accuracy, linearity, precision, robustness, LOD and LOQ.

Keywords: Hydrochlorothiazide, Amlodipine, Irbesartan, Atorvastatin, simultaneous, UPLC, validation

Introduction

Amlodipine, 3-ethyl 5-methyl-2-[(-2-(aminoethoxymethyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate (Fig.1), is a potent dihydropyridine calcium channel blocker used in the treatment of hypertension and angina pectoris[1,2] that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. it is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure[3]. Irbesartan 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-onean angiotensin II receptor blocker (Fig.2) is widely used for the treatment of hypertension. Angiotensin II is an octapeptide regarded as the main effectors of AT1 receptor in renin–angiotensin system. It causes vasoconstriction, tachycardia, and an increase of aldosterone secretion from the adrenal cortex and retention of sodium and body fluid[4]. Hydrochlorothiazide (HCTZ) 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, is an effective thiazide diuretic, (Fig.3) which has been used alone, or in combination with other antihypertensive drugs including β-blockers, ACE inhibitors, or angiotensin II receptor blockers for the treatment of hypertension and congestive cardiac failure[5,6]. Atorvastatin (AT) [*R*-(*R**, *R**)]-2-(4-fluorophenyl)-β,δ-dihydroxy- 5-(1-methylethyl)-3-phenyl-4[(phenyl amino) carbonyl]- 1*H*-pyrrole-1-heptanoic acid, is a member of the class of lipid-lowering agents called statins. (Fig.4) The drug is potent inhibitor of HMG-CoA reductase which inhibits the rate limiting enzyme in cholesterol biosynthesis. The present manuscript describes a simple, rapid, precise and accurate isocratic method for the simultaneous determination of ATV, IRBE, HCTZ and AMLO. The method may have industrial application as one method is sufficient for analyzing combination formulations and drugs individually.

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reduction of analysis time and solvent consumption by using a ACQUITY UPLC BEH C_{18} 2.1 x 150 mm, 1.7 µm column[7,8.]

Material and Methodology

Reagents and Chemicals

Working standard of Irbesartan and Hydrochlorothiazide were gifted by Ranbaxy Pvt. Ltd. (Dewas, India), Amlodipine was gifted by Sun pharmaceuticals Pvt. Ltd. (Silvassa, India) and Atorvastatin was gifted by Zydus Cadila Pvt. Ltd. (Ahmadabad, India), Analytical grade potassium dihydrogen phosphate, phosphoric acid and HPLC grade methanol, and acetonitrile were obtained from Merck (India).

Procedure for ultra-performance liquid chromatography Chromatographic conditions

UPLC method was performed by using a ACQUITY UPLC BEH C_{18} 2.1 x 150 mm, 1.7 µm column and a mobile phase consisting of 0.01 M potassium dihydrogen phosphate buffer : acetonitrile (70:30) (v/v) pH 5 while at a flow rate of 0.25 ml min^{-1} at room temperature.

Standards

Stock solutions of irbesartan, amlodipine, atorvastatin and hydrochlorothiazide were prepared by dissolving the appropriate amount of drug substance in ACN to yield a final drug concentration of 1.0 mg ml^{-1} respectively. dilutions were prepared for the calibration standards and further dilution were obtained by serial dilutions of stock solutions with ACN and get final concentration 10-60, 4-

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24, 5-25 and 4-24µg/ml for IRBE, AMLO, ATV, and HCTZ respectively.

Analysis of dosage form

Twenty tablets were weighed for each combination (Irbesartan or Hydrochlorothiazide, Irbesartan or Amlodipine and Irbesartan or Atorvastatin) their mean weight were determined and crushed in mortar. An amount of powdered mass equivalent to one tablet contents was transferred into a 50ml volumetric flask containing 10 ml of ACN, mechanically shaken for 10 min, ultrasonicated for 5 min, and then diluted to volume with mobile phase (sample stock solution). About 10 ml of sample stock solution was centrifuged at 10,000 rpm, and 5ml aliquot diluted to 50 ml with mobile phase (sample solution). A small portion of sample solution was filtered through 0.22µm PTFE filter and used for injection on UPLC.

Results and Discussion

UPLC Method development

Development of a UPLC method for simultaneous determination of irbesartan, hydrochlorothiazide, amlodipine and atorvastatin are simultaneously (Fig. 5)

The method has industrial application as one method is sufficient for analyzing three combination formulations. In the proposed work, a successful attempt has been made to develop simple, accurate and precise UPLC method for simultaneous estimation of that combination and also to validate it. By using ACQUITY UPLC BEH C_{18} 2.1 x 150 mm, 1.7 µm, column. And the mobile phases were used 0.01*M* potassium dihydrogen phosphate: acetonitrile (70:30) (v/v) pH 5 or flow 0.25 ml min^{-1}. Under the described experimental conditions the all peaks were well defined and free from tailing.

UPLC METHOD VALIDATION

The analytical method was validated with respect to parameters such as linearity, precision, accuracy, selectivity, recovery, Robustness, limit of quantitation (LOQ) and limit of detection (LOD), [9,11].The described method has been validated for assay and related substances by UPLC determination [12].

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 4-24 µg/ml for Hydrochlorothiazide, 4-24 µg/ml for Amlodipine, 5-30µg/ml Atorvastatin and 10-60µg/ml Irbesartan made separately. All the solutions were filtered through 0.22µm PTFE filter and injected, chromatograms were recorded, and it was repeated for six times. Correlation coefficients were found to be more than 0.999 for all four drugs.

Accuracy

Accuracy of the method was tested by spiking a mixture of three concentrations of the drug solutions and determining the percentage of recovery of added drug. The recovery of added standard (80%, 100%, and 120%) was found at three same concentration levels for each

% Recovery = Observed conc./Actual conc. **X** 100

drug. The mean of percentage recoveries (n = 9) and the relative standard deviation was calculated.(Tab.I)

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

The limit of detection and limit of quantitation were determined experimentally by using the signal to noise ratio method. The limit of detection was defined as the concentration that yields a signal to noise ratio of 3 and was found to be 1 ng /ml for Irbesartan, 1 ng/ml for Atorvastatin, 0.01 ng /ml Hydrochlorothiazide and 0.01µg/ml for Amlodipine

The limit of Quantitation was calculated to be the lowest concentration that could be measure with a signal to noise ratio of 10 and was found to be 0.01µg/ml for Irbesartan, 0.01µg/ml for Atorvastatin, 1 µg/ml for Amlodipine, 0.1ng/ml for Hydrochlorothiazide

Precision

The intra- and inter-day variability or precision data are summarized in Table II and Table III were assessed by performing replicate analysis of standard solutions in the mobile phase. Repeatability and reproducibility were characterized for different concentrations and given by mean recovery and %RSD. Intermediate precision was studied using different column, and performing the analysis on different day.

Intra day precision

Mix standards of irbesartan, hydrochlorothiazide, amlodipine and atorvastatin were prepared and analyzed in triplicates for Intraday precision.

Inter day Precision

Mix standard dilutions were prepared and its analysis was carried out in different days in different concentration and analysis for intermediate precision done. The results were validated statistically.

Robustness

The robustness as a measure of method capacity to remain unaffected by small, but deliberate changes in chromatographic conditions was studied by testing influence of small changes in pH of buffer (±0.2 units), change in column temperature, change in flow rate (±5%) and change in mobile phase ratio (±2 units). The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operators has concluded that the method is robust and the data are summarized in Table.4

Assay

A validated method was applied to determination of HTCZ, AMLO, IRBE and ATV in commercially available tablets. The result of the assays undertaken

yielded 100.02 %(%RSD=99.17%),99.98%(%RSD=0.222%),99.99%(% RSD=0.247%) and 99.97%(%RSD= 0.245%) of label claim for HCTZ, AMLO, IRBE and ATV respectively. The mean retention times of HCTZ, AMLO, IRBE and ATV were 2.9, 3.2, 3.6 and 6.1 min with associated %RSD values of 0.153%, 0.174%, 0.113% and 0.414% respectively. The results of assay indicate that the method is selective for the analysis of HCTZ, AMLO, IRBE and ATV of all four drugs without interference from the excipients used to formulate the tablets.

Conclusions

A simple, rapid, accurate and precise UPLC method for simultaneously determination of Irbesartan, Hydrochlorothiazide, Amlodipine and Atorvastatin was developed and validated for the routine analysis of all four drugs in API and tablet dosage forms. The proposed method gives good resolution between selected compounds. There was no significant difference for the assay tested with in-day and between-day. The method developed would serve as a versatile analytical tool suitable for the analysis of these drugs and would be of interest for quality control and clinical monitoring laboratories. High percentage recovery shows that the methods are free from the interferences of the endogenous substances in tablet form.

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Table.I Recovery studies (n=3) separately

parameter	HCTZ	AMLO	IRBE	ATV
Mean Percent recovery	99.17	99.03	99.05	98.84
S.D	0.3960	0.2205	0.2453	0.2430
%R.S.D	*0.399*	0.222	*0.247*	0.245

***Table.II* Intraday precision data for precision of irbesartan, hydrochlorothiazide, amlodipine and atorvastatin (n=3) separately**

S.NO.	Name of Drugs	S.D.	% RSD
1	Irbesartan	0.0863	0.0864
2	Hydrochlorothiazide	0.7404	0.740
3	Amlodipine	0.2724	0.272
4	Atorvastatin	0.1821	0.182

Table.III Interday Precision (n=3) separately

Drugs name	Mean	S.D.	%R.S.D.
Irbesartan	99.66	0.1435	0.143
Hydrochlorothiazide	99.89	0.095	0.0951
Amlodipine	99.68	0.478	0.479
Atorvastatin	99.9	0.041	0.0400

Table.IV **Robustness testing of the method**

parameter	Modification in A:B(70:30)		HCTZ	AMLO	IRBE	ATV
Mobile Phase A: B ratio	68:32	S.D.	0.283	0.2867	0.1466	0.2232
		%R.S.D	0.285	0.287	0.146	0.224
	72:28	S.D.	0.9964	0.6547	0.1404	0.2074
		%R.S.D	0.996	0.661	0.140	0.207
pH(5.0)	4.8	S.D.	0.2287	0.6217	0.0772	0.5157
		%R.S.D	0.229	0.628	0.077	0.518
	5.2	S.D.	0.2320	0.6403	0.2767	0.5548
		%R.S.D	0.233	0.647	0.277	0.557
Flow rate (0.25)	0.23	S.D.	0.8036	0.6932	0.1438	0.1603
		%R.S.D	0.804	0.699	0.144	0.160
	0.27	S.D.	0.2767	0.6403	0.2767	0.5548
		%R.S.D	0.281	0.647	0.277	0.557

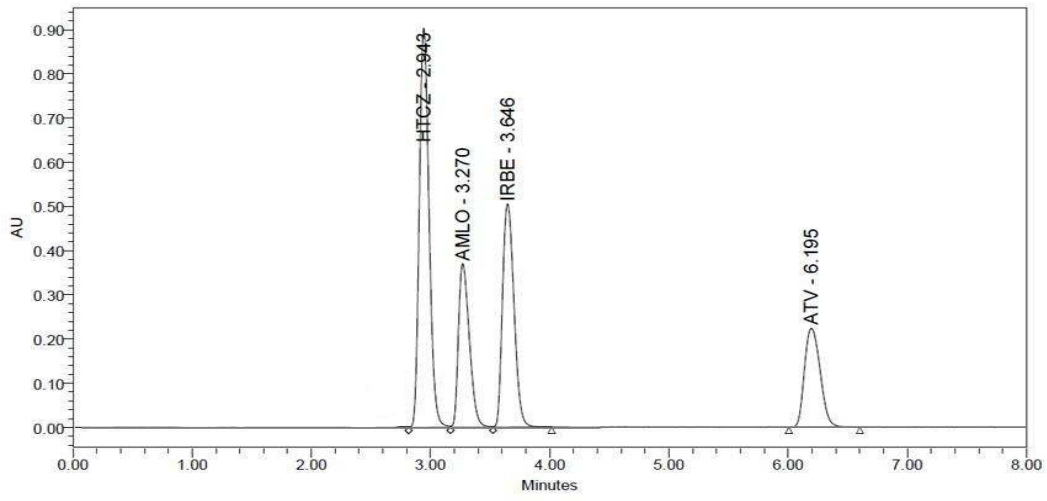

Figure.5 Chromatogram of mix standard of Hydrochlorothiazide, Amlodipine, Irbesartan and Atorvastatin

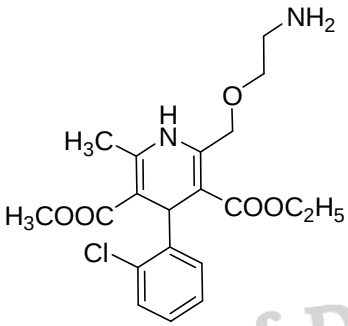

Figure.1 Amlodipine

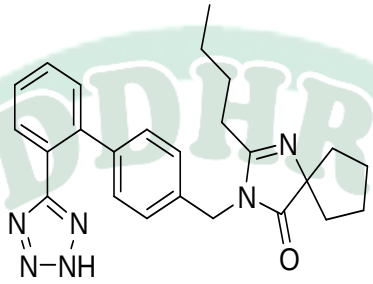

Figure .2 Irbesartan

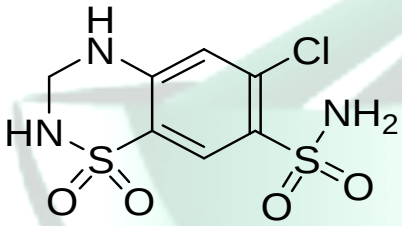

Figure .3 Hydrochlorothiazide

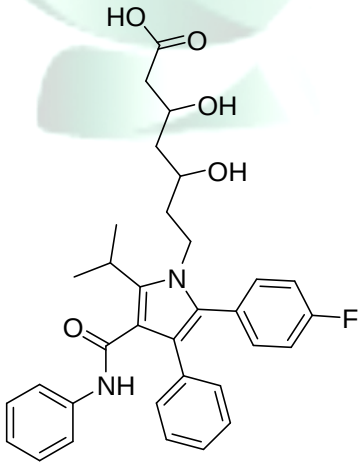

Figure. 4 Atorvastatin

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