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HIGH RESOLUTION RP-HPLC METHOD FOR THE DETERMINATION OF HYPERTENSIVE DRUG PRODUCTS

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ABSTRACT

OBJECTIVE: To develop a RP-HPLC method for the simultaneous determination of eight active ingredients including hydrochlorothiazide (HCTZ), metoprolol succinate, valsartan, losartan Potassium, benazepril hydrochloride, telmisartan, amlodipine besylate and atorvastatin in pharmaceutical products. METHOD: HPLC analysis was carried out by using a XTerra C18 column with the gradient mobile phase composed of Sol-A: ammonium acetate buffer (1.4g of ammonium acetate in to 1000ml of HPLC water) and sol-B: acetonitrile with simple gradient program (0-5min, sol-A:88-85; 5-10min- sol-A:85-70; 10-18min- sol-A:70-70; 18-25min- sol-A:70-55; 25-30min- sol-A:55-88 and 30-35min- sol-A:88-88). Flow for mobile phase elution is 1.0ml per min; column oven temperature is maintained at 35°C and record the absorbance with 230nm.RESULT: The co-relation coefficient for all eight active ingredients is not less than 0.999. The average recoveries of the all components were 98.0% to 102.0%.CONCLUSION: This HPLC method is simple, quick and reproducible, with high recovery and has been successfully applied to the simultaneous determination of the eight components in pharmaceutical drug products.

KEY WORDS: Hydrochlorothiazide (HCTZ), Metoprolol succinate, Valsartan, Losartan Potassium, Benazepril hydrochloride, Telmisartan, Amlodipine besylate, Atorvastatin, Hypertensive drugs, Cardiovascular drugs and RP-HPLC method development and validation.

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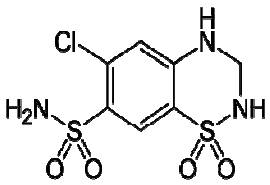
INTRODUCTION

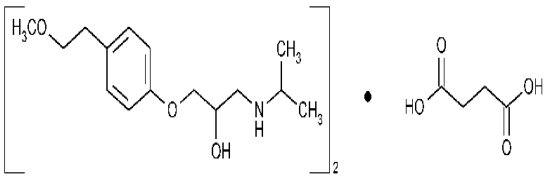
Pharmaceutical products formulated with one drug or multiple drugs, typically referred to as combination products. These combination products require accurate and precise qualitative and quantitative analytical methods for the determination of each active ingredient. This present research work is to develop a single RP-HPLC method for all eight (hydrochlorothiazide (HCTZ), metoprolol succinate, valsartan, losartan Potassium, benazepril hydrochloride, telmisartan, amlodipine besylate and atorvastatin) hypertensive drug products. Amlodipine is a calcium channel blocker that inhibits the trans membrane influx of calcium ions into vascular smooth muscle and cardiac muscle and used for anti-hypertension and angine pectoris (chest pain). Amlodipine is a chiral calcium antagonist, in therapeutic use as a racemate [1,2]. The recommended dose for adults is 5-10 mg once daily and pediatric patients is 2.5 mg to 5 mg once daily[3, 4]. Metoprolol succinate used in treatment of several diseases of the cardiovascular system, especially hypertension. The active substance metoprolol is employed either as metoprolol succinate or metoprolol tartrate. Losartan Potassium [5 to 8] is an angiotensin II receptor antagonist. And used for the treatment of high blood pressure (hypertension). The recommended dosage of losartan-hydrochlorothiazide for people with high blood pressure (hypertension) will vary between losartan-hydrochlorothiazide 50 mg/12.5 mg and losartan-hydrochlorothiazide 100 mg/25 mg once a day. Losartan potassium and doxycycline combined completely prevented thoracic aortic aneurysm and improved elastic fiber organization [9].

Atorvastatin [10-13] is a statins class of drug, used for lowering blood cholesterol. It prevents strokes through anti-inflammatory and other mechanisms. Atorvastatin comes as a tablet to take by mouth. It is usually taken once a day with or without food. Atorvastatin may cause side effects. Hydrochlorothiazide (HCTZ) [14, 15] is a diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart. Hydrochlorothiazide is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and the prevention of kidney stones. The recommended dose of hydrochlorothiazide for treating high blood pressure is hydrochlorothiazide 25 mg to 50 mg per day. enazepril [16-19] used for the treatment of high blood pressure (hypertension), congestive heart failure and chronic renal failure. The available oral tablets are 5 mg, 10 mg, 20 mg and 40 mg. Benazepril is also available in combination with hydrochlorothiazide. Telmisartan [20, 21] is an angiotensin II receptor antagonist used in the management of hypertension. The usually effective dose telmisartan is (20–) 40–80 mg once daily. Some patients may already benefit at a daily dose of 20 mg. In cases where the target blood pressure is not achieved, telmisartan dose can be increased to a maximum of 80 mg once daily. Valsartan [22-26] is an angiotensin-II receptor antagonist. Valsartan is indicated for treatment of high blood pressure, congestive heart failure (CHF) or post-myocardial. Oral tablets, containing 40 mg, 80 mg, 160 mg or 320 mg of valsartan. Usual dosage ranges from 40–320 mg daily. The chemical structure of the all active ingredients represented in figure-1.

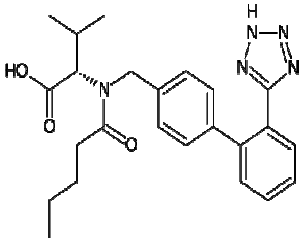
Hydrochlorothiazide (Hctz)



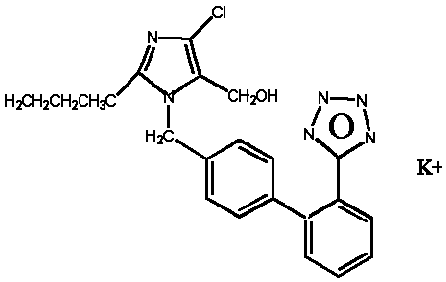
Metoprolol Succinate



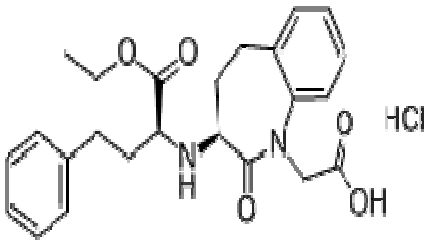
Valsartan



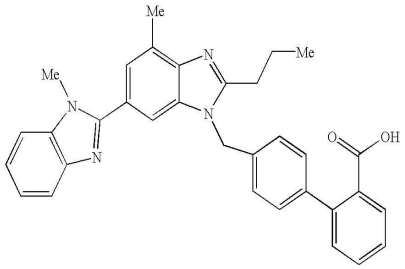
Losartan Potassium



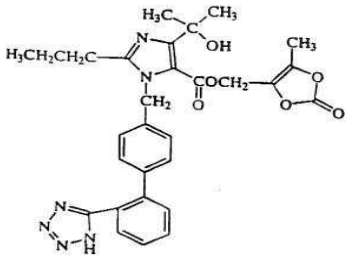
Benazepril Hydrochloride



Telmisartan



Amlodipine Besylate



Atorvastatin

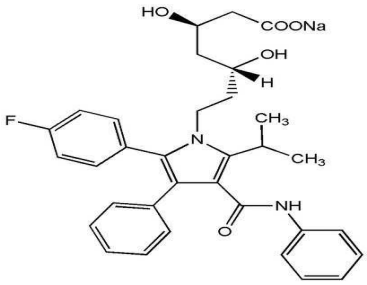


Figure-1
Chemical structure of active ingredients

All eight ingredients (hydrochlorothiazide (HCTZ), metoprolol succinate, valsartan, losartan Potassium, benazepril hydrochloride, telmisartan, amlodipine besylate and atorvastatin) are available in individual and combination dosage forms. All combination products have individual methods [27 to 46]. The available products in market are mentioned in the table-1.

Table-1
Available dosage forms in market.

Dosage form	Active ingredients
Tablets	Amlodipine Besylate -25mg, 5mg and 10mg
Tablets	Losartan Potassium -25mg and 50mg
Tablets	Metoprolol succinate -50mg and 100mg
Injection	Metoprolol succinate-1mg/ml
Tablets	Benazepril HCl-5mg, 10mg and 20mg
Tablets	Atorvastatin-10mg and 20mg
Tablets	Telmisartan-20mg, 40mg and 80mg
Tablets	Valsartan-40 mg, 80 mg, 160 mg or 320 mg
Tablets	Valsartan-80 mg and Amlodipine-2.5mg
Tablets	Hydrochlorothiazide-12.5mg and Valsartan-, 80 mg or 160 mg
Tablets	Amlodipine Bisylate - 5mg
	Losartan Potassium - 50mg
Tablets	Losartan Potassium - 50mg
	Hydrochlorothiazide-12.5mg
Tablets	Amlodipine Besylate -5mg
	Metoprolol succinate -50mg and 100mg
Tablets	Metoprolol succinate -100mg
	Hydrochlorothiazide-12.5mg
Capsules	Benazepril HCl and Amlodipine Besylate -2.5mg/10mg and 10mg/40mg
Tablets	Telmisartan-40mg
	Amlodipine-2.5mg
Tablets	Amlodipine Besylate -0.5mg
	Losartan Potassium -50mg
	Hydrochlorothiazide-12.5mg
Tablets	Amlodipine Besylate and Atorvastatin-2.5mg/10mg, 2.5mg/20mg, 2.5mg/40mg and (multiple strengths)

Developed a high resolution RP-HPLC method for the above eight active ingredients including hydrochlorothiazide (HCTZ), metoprolol succinate, valsartan, losartan Potassium, benazepril hydrochloride, telmisartan, amlodipine besylate and atorvastatin in pharmaceutical products and validated the method with specificity, linearity, accuracy and reproducibility.

MATERIALS AND METHODS

Apparatus and Chromatographic conditions
Chromatographic separation was achieved for all actives on a Water make chromatographic system equipped with an Alliance 2695 module and Agilent HPLC module 1200, variable wavelength programmable UV/Visible detector and Mettler Toledo analytical balance. Injection volume is 20µL and XTerra RP-18 HPLC column (150 × 4.6 mm, 3.5micron) was used for separation. Mobile phase consisting of Sol-A: 1.4g of ammonium acetate in 1000ml of HPLC water, Sol-BL acetonitrile with simple gradient elution (0-5min, sol-A:88-85; 5-10min-sol-A:85-70; 10-18min- sol-A:70-70; 18-25min-sol-A:70-55; 25-30min- sol-A:55-88 and 30-35min- sol-A:88-88) was delivered at a flow rate of 1.0ml per min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15min. Analysis was performed at 35°C temperature. A mixture of water and acetonitrile (1:1) used as diluents for analysis.

Reagents and Solutions
Pure (not less than 98.5%) sample of all active ingredients standards (hydrochlorothiazide

(HCTZ), metoprolol succinate, valsartan, losartan Potassium, benazepril hydrochloride, telmisartan, amlodipine besylate and atorvastatin), HPLC grade acetonitrile and water were used. All other reagents used in this study were of AR grade.

Standard solution

The solution was weighed accurately 40mg of all active ingredient standards, transferred into 100 ml of volumetric flask and sonicated for 15min and diluted to 100ml volume with diluent. Furtherdiluted the above solution to get the 40ppm of each ingredient.

Sample solution

Weighed, crushed the tablets, capsules and weighed the crushed powder equivalent to 40mg of each ingredient and prepared the solution to get the known concentration of 40ppm for all ingredients.

Calculation

All eight active ingredients are quantified with the following calculation.

$$\frac{\text{Sample area x standard dilution factor}}{\text{Standard area x sample dilution factor}} \times 100$$

RESULTS AND DISCUSSIONS

Method development

The goal of this work is to develop a single RP-HPLC method to determine all eight APIs (hydrochlorothiazide (HCTZ), metoprolol succinate, valsartan, losartan Potassium, benazepril hydrochloride, telmisartan, amlodipine besylate and atorvastatin) in the individual and combination drug products. During the early stage of the method development trials done with phosphate buffer, acetonitrile as solvent and C18 column but the elution of amlodipine and atorvastatin is late and peak shape is poor. Finally the separation was achieved with ammonium acetate buffer and acetonitrile with simple gradient program. Diluent and standard solution represented in figure-1 and 2. All the active ingredients are well separated and the peak shape, resolution (not less than 3.0) and tailing factor (less than 1.5) also within the limit.

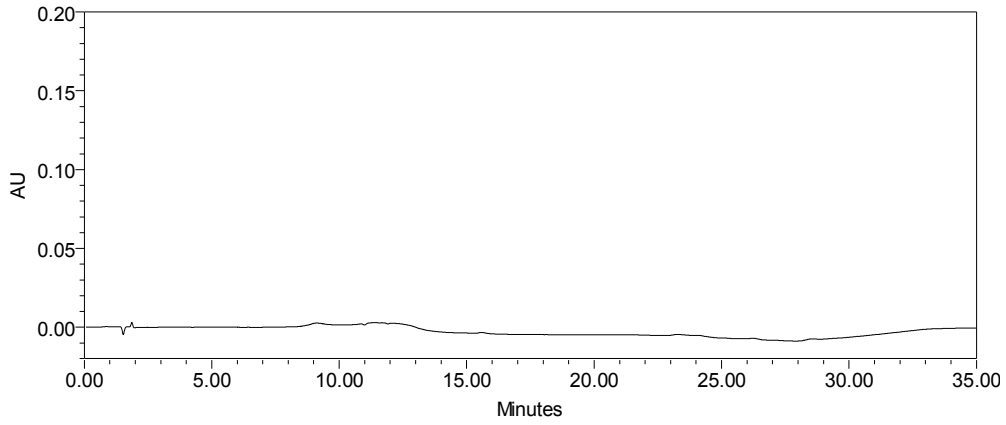


Figure-1
Diluent chromatogram

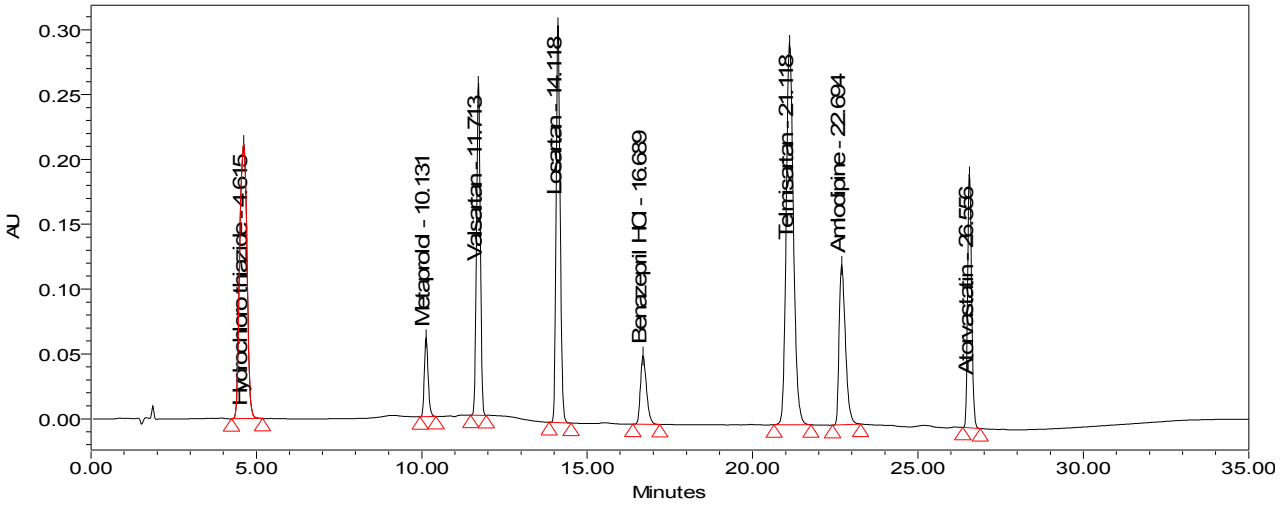


Figure-2
Standard chromatogram

System suitability

System suitability testing is an integral part of method development of analytical procedures. System suitability test parameters are established for the developed method. Freshly prepared standard solution (each active 40ppm) in to the system for five replicate injections and calculated the percentage relative standard deviation for area and retention time and the results found to be satisfactory. Five replicate standard solution chromatograms represented in figure-3 and tabulated the results in table-2 and 3.

Table-2
System suitability (Area %RSD)

Active Ingredient Name	Standard solution Area						
	Injection-1	Injection-2	Injection-3	Injection-4	Injection-5	Average	%RSD
Hydrochlorothiazide	3234625	3226357	3224662	3216728	3226777	3225830	0.20
Metoprolol succinate	486800	487785	486078	484181	486339	486236	0.27
Valsartan	2044041	2045973	2046665	2049147	2052272	2047620	0.16
Losartan Potassium	2609713	2610103	2610273	2612470	2616056	2611723	0.10
Benazepril HCl	668485	668486	666506	670274	671825	669115	0.30
Telmisartan	4475851	4474466	4470447	4490813	4473945	4477104	0.18
Amlodipine besylate	1625730	1619985	1624407	1624384	1619153	1622732	0.18
Atorvastatin	1626606	1640399	1624204	1626498	1592514	1622044	1.09

Table-3
System suitability (Retention time %RSD)

Active Ingredient Name	Standard solution Retention time (min)						
	Injection-1	Injection-2	Injection-3	Injection-4	Injection-5	Average	%RSD
Hydrochlorothiazide	4.61	4.61	4.6	4.61	4.6	4.606	0.12
Metoprolol succinate	10.13	10.13	10.12	10.12	10.11	10.122	0.08
Valsartan	11.71	11.7	11.69	11.7	11.68	11.696	0.10
Losartan Potassium	14.11	14.1	14.09	14.09	14.08	14.094	0.08
Benazepril HCl	16.68	16.66	16.65	16.65	16.63	16.654	0.11
Telmisartan	21.11	21.07	21.06	21.06	21.02	21.064	0.15
Amlodipine besylate	22.69	22.68	22.67	22.68	22.66	22.676	0.05
Atorvastatin	26.55	26.53	26.52	26.53	26.51	26.528	0.06

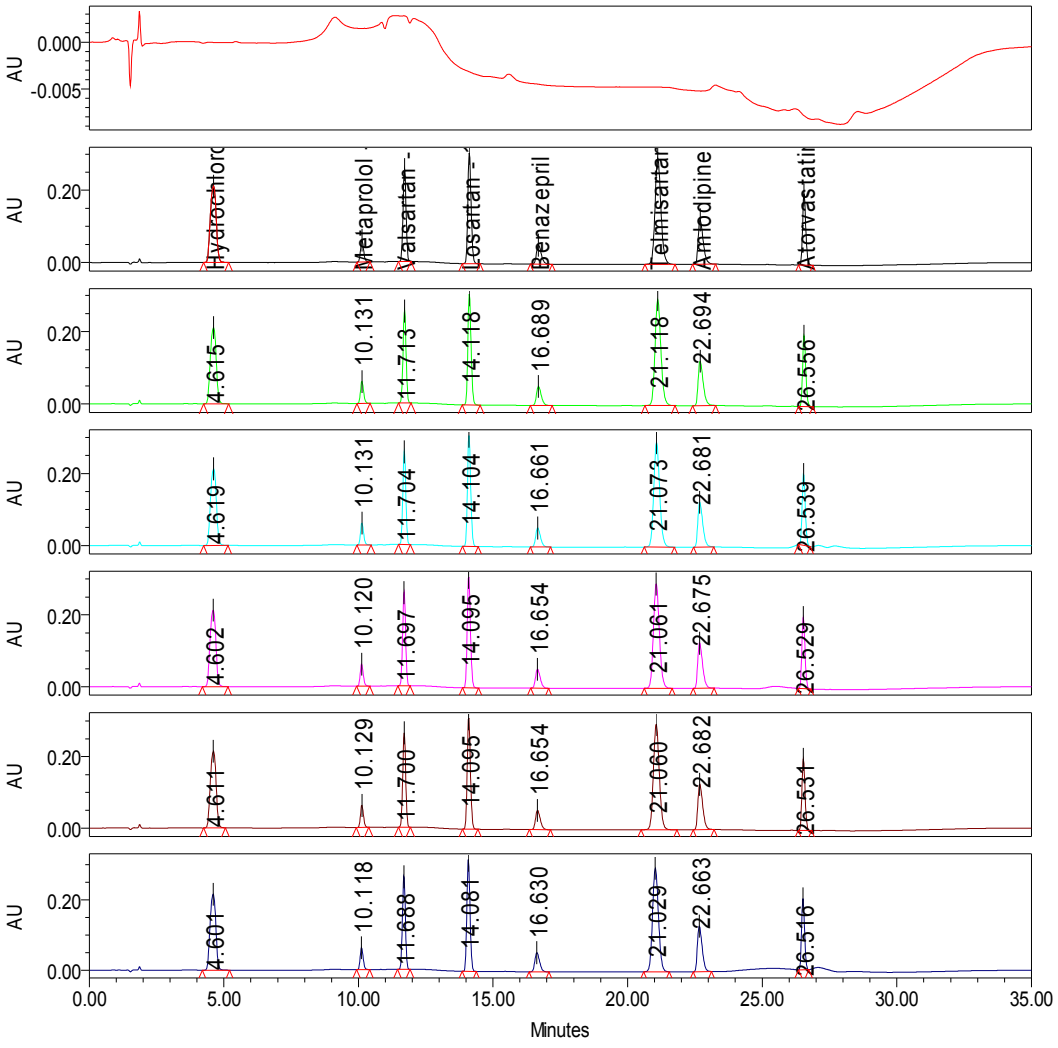


Figure-3
System suitability chromatograms

Method validation

Once the HPLC method development was over, validated the developed method as per ICH and FDA [47- 50] guidelines with parameters like specificity, precision, accuracy, linearity and range, ruggedness, robustness etc.

Specificity

Condition of HPLC method like column temperature, ionic strength of buffer and flow rate etc, was changed. In spite of above changes no additional peaks were found, although there were shift retention times or little changes in peak shapes but the system suitability and precision results found to be good and no interference was observed with diluent and placebo. Diluent and standard overlaid chromatograms represented in figure-4A and 4B.

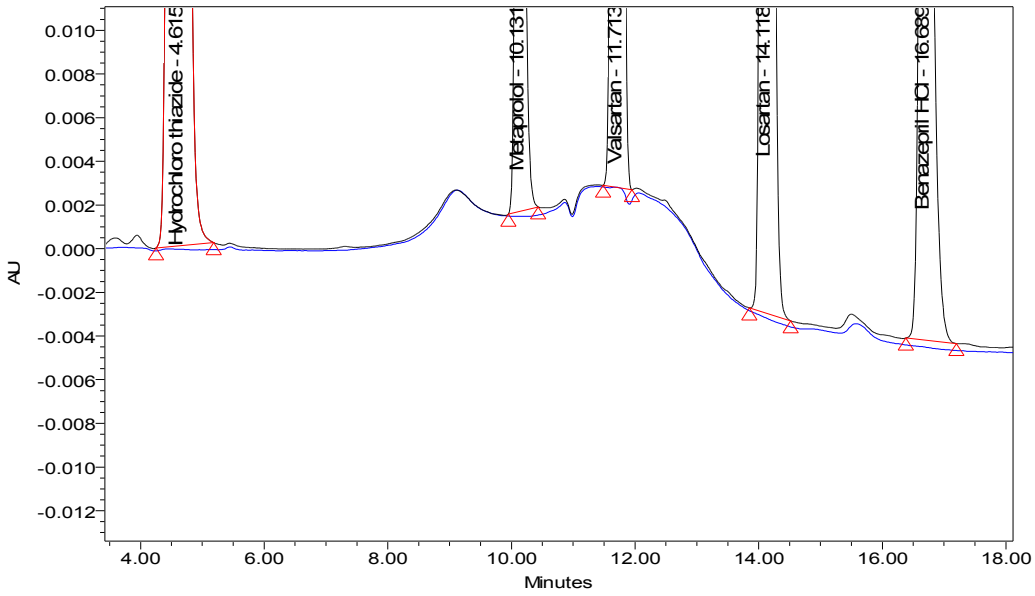


Figure-4A:
Diluent and standard overlaid chromatograms (zoom).

Precision

Precision was evaluated by carrying out six different sample preparations for all individual and combination products. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that that method is precise. Results were shown in Table-4.

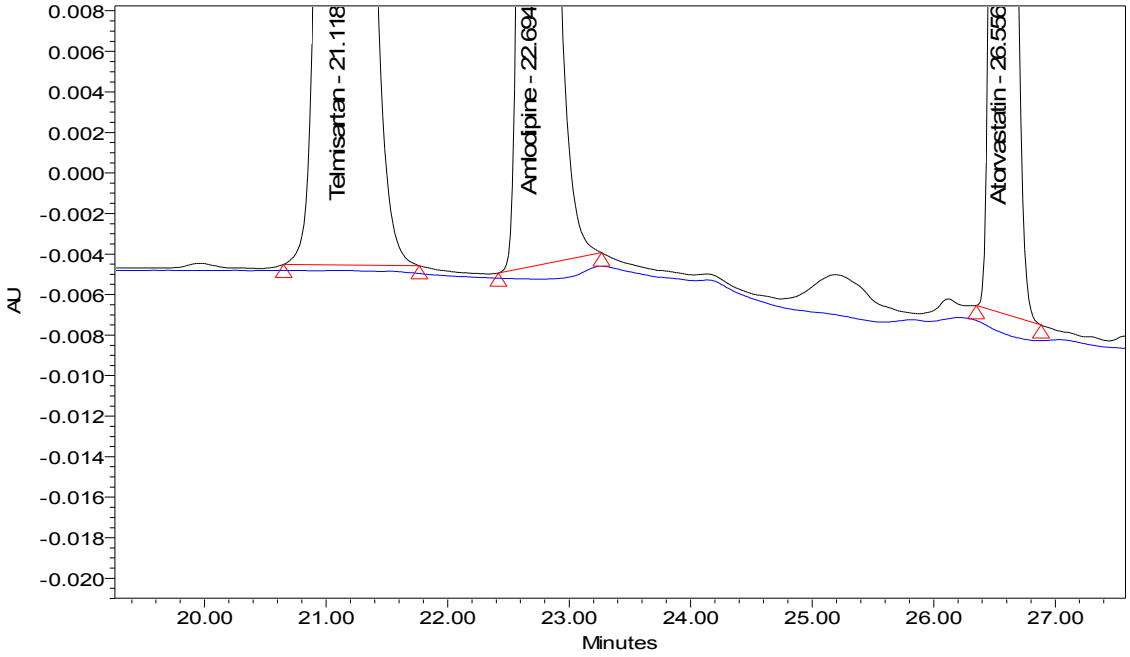


Figure-4B
Diluent and standard overlaid chromatograms (zoom).

Table-4
Precision Results.

Active Ingredient Name	Sample preparations						Average (%)
	Prep-1	Prep-2	Prep-3	Prep-4	Prep-5	Prep-6	
Hydrochlorothiazide	99.41	99.80	99.36	98.70	98.85	97.90	99.00
Metoprolol succinate	100.50	97.90	100.34	99.09	100.10	100.50	99.74
Valsartan	100.40	100.50	100.58	99.18	99.81	101.52	100.33
Losartan Potassium	99.78	99.92	100.50	99.78	99.24	99.63	99.81
Benazepril HCl	100.85	99.74	101.63	99.58	99.86	99.27	100.16
Telmisartan	99.81	99.27	100.51	99.97	99.70	99.25	99.75
Amlodipine besylate	98.89	98.70	101.23	98.89	98.12	99.01	99.14
Atorvastatin	101.52	97.98	102.01	101.20	98.85	100.50	100.34

Linearity

The linearity of method was evaluated by analyzing different concentrations of the standard solution (mixture of all active ingredients). Calibration curve was constructed by plotting area against concentration and regression equation was computed. The results were shown in table-5. The correlation coefficient value found to be within the limit 0.999. The linearity chromatograms shown in figure-5 and linearity results tabulated in table-5.

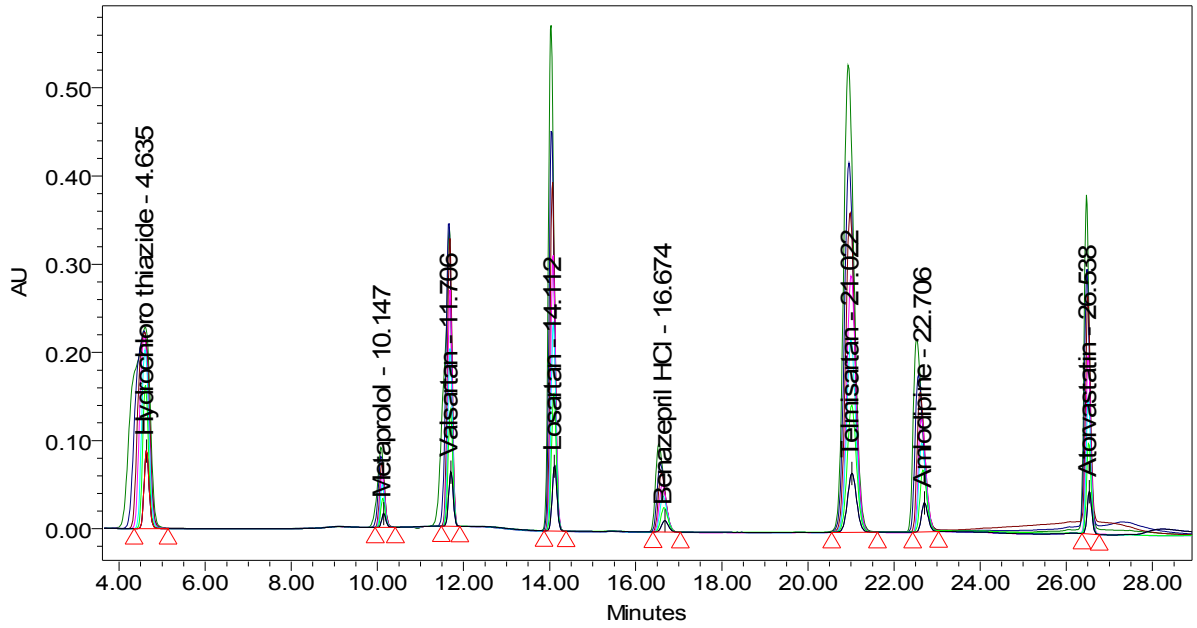


Figure-5
Linearity chromatograms

Table-5
Linearity Results.

Active Ingredient Name	Linearity solutions area						
	Level-1 (10ppm)	Level-2 (20ppm)	Level-3 (30ppm)	Level-4 (40ppm)	Level-5 (50ppm)	Level-6 (60ppm)	Co-relation Coefficient
Hydrochlorothiazide	728425	1563019	2393003	3220184	4042337	4872147	0.999998
Metoprolol succinate	108264	235385	361778	487608	612792	739466	0.999997
Valsartan	458859	992341	1526878	2060094	2588104	3125365	0.999998
Losartan Potassium	583214	1264843	1945574	2626700	3300832	3983219	0.999999
Benazepril HCl	148973	324291	498776	673285	847021	1021894	0.999999
Telmisartan	1000831	2165010	3328933	4488875	5643699	6807496	0.999999
Amlodipine besylate	355150	776119	1200659	1625057	2044851	2470964	0.999999
Atorvastatin	360630	784942	1210756	1633540	2062646	2480960	0.999997

Accuracy

To study the reliability and accuracy of the method recovery experiments were carried out. A known quantity of the pure drug was added to the placebo sample at the level of 25% and 100% of the test concentration. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The mean recoveries were in range of 98.0-102.0 % which shows that there is no interference from excipients. Table-6 represents the recovery results.

Table-6
Accuracy (recovery) Results.

Active Ingredient Name	Spike level						Average % Recovery
	25%	50%	75%	100%	125%	150%	
Hydrochlorothiazide	99.41	99.70	99.36	98.70	99.01	100.60	99.46
Metoprolol succinate	100.50	98.12	100.34	99.09	100.50	99.80	99.73
Valsartan	100.40	98.85	100.58	99.18	100.34	99.23	99.76
Losartan Potassium	99.00	99.92	99.63	99.78	99.24	100.82	99.73
Benazepril HCl	99.74	97.90	99.27	99.58	99.86	101.20	99.59
Telmisartan	100.33	100.50	99.25	99.97	99.70	100.62	100.06
Amlodipine besylate	99.81	101.52	101.23	98.89	98.12	99.84	99.90
Atorvastatin	100.16	97.98	102.01	101.20	98.85	98.60	99.80

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like waters HPLC and Agilent HPLC by different operators using different columns of similar types. The percentage RSD of six different preparations assay values with two different instruments, analysts and columns were 0.5- 0.5, 0.6- 0.5 and 0.4- 0.3% respectively. Robustness of the method was determined by making slight changes in the chromatographic conditions, such as flow rate and column temperature. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust. The robustness limit for mobile phase variation, flow rate variation and temperature variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria. Robustness results were tabulated in table-7.

Table-7
Robustness Results.

Parameter	System suitability		
	Variation	Tailing factor	Percent (%) RSD
Standard solution	-----	1.1-1.3	1.2-1.5
Flow Rate	+0.1mL per min	0.8-1.0	0.8-1.2
	-0.1mL per min	0.9-1.1	0.9-1.2
Column Oven Temperature	+5°C	1.1-1.5	1.0-1.2
	-5°C	1.2-1.4	1.1-1.3

CONCLUSION

The proposed reversed phase liquid chromat- -ographic method was validated with linearity, precision, accuracy and specificity and proved to be convenient and effective for the determination of all active ingredients during stability testing of the bulk as well as pharmaceutical tablet dosage form. This method is applicable for all eight ingredients (hydrochlorothiazide (HCTZ), metoprolol succinate, valsartan, losartan Potassium, benazepril hydrochloride, telmisartan, amlodipine besylate and atorvastatin) in pharmaceutical drug products. The developed method has potential application for all

ingredients and applicable for routine quality control analysis. Moreover, the single method, lower solvent consumption along with the short analysis time leads to cost effective chromatographic method.

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