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SIMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE, AMLODIPINE BESYLATE AND TELMISARTAN IN COMBINED TABLET DOSAGE FORM BY USING RP- HPLC METHOD

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Keywords:

RP-HPLC, Hydrochlorothiazide, Amlodipine besylate, Telmisartan, Validation

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ABSTRACT: A simple, selective, rapid, precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous estimation of Hydrochlorothiazide, Amlodipine besylate and Telmisartan in tablet dosage form as per ICH guidelines. The wavelength selected for the analysis was 239 nm for amlodipine besylate, telmisartan and hydrochlorothiazide. The solvents used were methanol and distilled water. The mobile phase 0.05M Potassium dihydrogen ortho-phosphate (pH-3.2), acetonitrile and methanol in the ratio 45:45:10 was used to carry out the separation. Column used was Supelco C18 (250 x 4.6 mm, 5µ). Flow rate was selected as 1ml/min. Retention time for Hydrochlorothizide (HCT), Amlodipine besylate (AML) and Telmisartan (TEL) was found to be 2.9min., 5.1min., 8.2min. The developed method was validated for accuracy, precision, linearity, robustness, ruggedness, specificity. The proposed method can be used for the estimation of these drugs in combined dosage forms.

INTRODUCTION: The chemical name for Amlodipine besylate is 3-ethyl 5-methyl 4RS-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl - 1, 4-dihydropyridine - 3, 5-dicarboxylate benzene sulphonate. [1] Amlodipine besylate is the Calcium channel blocker. [2] It is used as an anti-hypertensive and in the treatment of angina. Amlodipine besylate is a white crystalline powder. It is slightly soluble in water and sparingly soluble in ethanol. It is official in BP, EP, IP, USP.[1, 3, 4, 5]

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Methods available for the determinations of AML include UV spectroscopy [6, 7, 8], High performance liquid chromatography [9, 10, 11], High performance thin layer chromatography [12, 13], LC-MS [14], LC - MS/ MS [15], and stability indicating assay method.[16] The chemical name for HCT, 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine - 7 - sulphonamide 1, 1-dioxide. It is a thiazide diuretic.[17] It is also used in the treatment of hyper-tension.[18]

HCT is White to off-white crystalline powder.[19] Soluble in dilute ammonia, or sodium hydroxide; also soluble in methanol, ethanol, acetone.[20] It is official in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia. [21, 17, 22] Methods available for determination of HCT includes spectrophotometry [23,] liquid chromatography [24, 25], stability indicating assay [26]

method and thin-layer chromatography [27], as alone or in combination with some other drugs.

Telmisartan is is chemically 4′-[(1,4′-dimethyl-2-propyl [2,6′-bi-1*H*-benzimidazol]-1′-yl) methyl] [1,1′-biphenyl]-2-carboxylic acid. [28] It is an Antihypertensive drug. [29] This is used for treatment of hypertension and diabetic nephropathy. [30, 31]

It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and U.S. Pharmacopoeia (USP).[32, 33, 34]

Methods for determination of Telmisartan includes UV, [35, 36, 37] visible spectrophotometric, [38] Colorimetry [39, 40], liquid chromatography-tandem mass spectrometry [41, 42, 43, 44] and HPTLC.[45, 46]

MATERIALS AND METHODS:

Instrumentation: Shimadzu HPLC system with autosampler (model LC-2010-HT) having UV detector and column heater was used. Data collection and analysis were performed using LC Solution software. Other instruments used are mentioned in the **Table 1.**

TABLE 1: INSTRUMENTS USED FOR METHOD DEVELOPMENT

Sr. No.	Name of the instrument	Company
1	HPLC	Shimadzu
2	Weighing balance	Premier weighing system
3	pH meter	DBK Digital pH meter
4	Sonicator	Oscar

Reagents & Chemicals: Pharmaceutically pure sample of AML, TEL and HCT were obtained as a gift samples from Smruti Organics; Glenmark Pharmaceuticals; IPCA Laboratories respectively. All solvents were of HPLC grade. A combination of AML (5 mg), TEL (40 mg) and HCT (12.5 mg) in tablet formulation was purchased from local market. (TELVAS-3D; Aristo Pharmaceuticals). All Reagents and chemicals used for method development are given in **Table 2**.

TABLE 2: REAGENTS AND CHEMICALS

Name of Chemicals	Grade	Manufacturer
Acetonitrile	HPLC & Analytical	Pallav Chemicals
Methanol	HPLC & Analytical	Pallav Chemicals
Potassium Dihydrogen Orthophosphate	Analytical	LOBA Chemie
O-Phosphoric Acid	Analytical	Pallav Chemicals
Distilled Water	HPLC	MilliQ Water

Chromatographic conditions: Column- Supelco C18 (250×4.6mm×5µ) was used for the separation. Mobile phase of buffer 0.05M sodium dihydrogen phosphate with pH 3.2, acetonitrile and methanol were mixed in the ratio 45:45:10, filtered through 0.45µ membrane filter, degassed and used for the separation. The flow rate was 1ml/min and inject volume was 20µL with detection at 239nm and analysis was performed. Chromatographic conditions are mentioned in the **Table 3**.

TABLE 3: CHROMATOGRAPHIC CONDITIONS

Mobile phase	Buffer(pH 3.2): ACN: Methanol (45: 45: 10 v/v)
Column	Supelco C18, 250 x 4.6 mm, 5µ
Column Temperature	30°C
Wavelength	239 nm
Injection Loop	20µl
Flow rate	1.0ml/min
Run Time	10mins
Elution	Isocratic

Preparation of Solutions:

a. Preparation of Buffer Solution pH 3.2: Weigh accurately 6.8 gm of potassium dihydrogen phosphate and mixed it with 1000ml of water. Adjust the pH to 3.2 ± 0.05 with ortho- phosphoric acid and filter through 0.45µ nylon membrane and degas.

b. Preparation of Diluent: Prepare a mixture of Water and Methanol in the ratio 50:50v/v respectively and mix.

c. Preparation of Standard Solution: Weigh accurately about 12.5 mg of Hydrochlorothiazide, 40 mg of Telmisartan, 5mg of Amlodipine besylate API and transfer it into 100ml volumetric flask. Add about 70ml of diluent and sonicate to dissolve, then cool and make up to the mark with diluent and mix. Further dilute 1 ml of this solution into 10 ml volumetric flask and make up to the mark with diluent and mix. (Standard Solution 12.5µg/ml HCT, 40µg/ml TEL, 5µg/ml AML).

d. Sample preparation: Weigh and transfer 10 tablets into 100ml volumetric flask. Add about 70ml of Diluent and sonicate with intermittent shaking. Allow it to cool at room temperature and make up to the mark with Diluent and mix. Further, dilute 1ml of that solution to 100ml with diluent and mix. Filter the sample solution through 0.45µ Nylon filter. Discard first 2ml of filtrate.

RESULTS AND DISCUSSION: Before validation of the developed method, analysis of blank solution (only mobile phase), standard solution (mixed standard) and sample solution (tablet mixture) was carried out. Following were the results obtained.

Blank Solution:

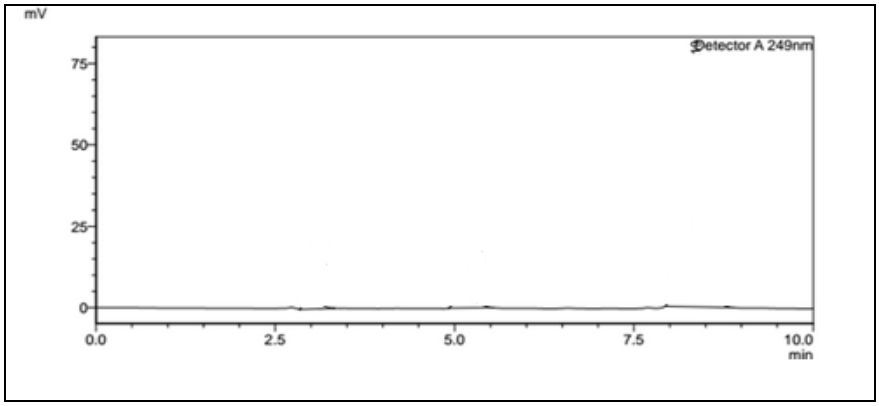

FIG.1: CHROMATOGRAM OF BLANK SOLUTION

Observation: It was found that the blank solution does not show any interference of characteristic peak and therefore can be used for estimation of drugs.

Standard Solution:

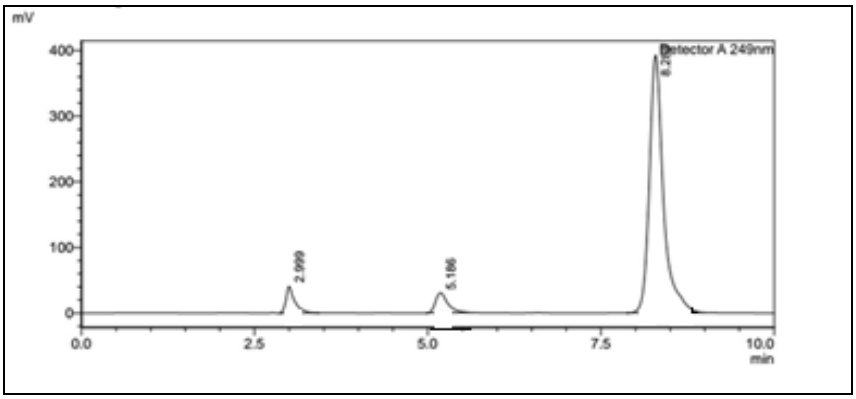

FIG. 2: CHROMATOGRAM OF STANDARD SOLUTION 12.5µg/ml HCT, 40µg/ml TEL, 5µg/ml AML

Sample Solution:

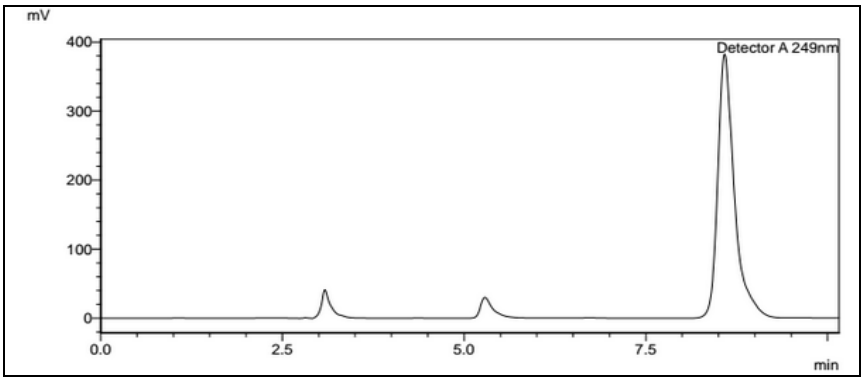

FIG. 3: CHROMATOGRAM OF SAMPLE SOLUTION 12.5µg/ml HCT, 40µg/ml TEL, 5µg/ml AML

The method validation was done according to the ICH guidelines. The following validation characteristic parameters are accuracy, precision, linearity, robustness, ruggedness and specificity.

1. Accuracy: The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in **Table 4, 5** and **6.**

TABLE 4: SERIES OF ACCURACY SAMPLES PREPARED OF HCT FOR ASSAY METHOD

Recovery Level %	Amount of Sample Added (µg/ml)	Amount of Standard Added (µg/ml)	Total concentration (µg/ml)	Mean peak area	% Recovery	% RSD
80	5	6.25	11.25	355729	101.35	0.70
100	6.25	6.25	12.5	352640	100.47	0.55
120	7.5	6.25	13.75	354290	100.94	1.02

TABLE 5: SERIES OF ACCURACY SAMPLES PREPARED OF AML FOR ASSAY METHOD

Recovery Level %	Amount of Sample Added (µg/ml)	Amount of Standard Added (µg/ml)	Total concentration (µg/ml)	Mean peak area	% Recovery	% RSD
80	2	2.5	4.5	371044	100.78	1.09
100	2.5	2.5	5	369129	100.26	0.94
120	3	2.5	5.5	371780	100.98	0.75

TABLE 6: SERIES OF ACCURACY SAMPLES PREPARED OF TEL FOR ASSAY METHOD

Recovery Level %	Amount of Sample Added (µg/ml)	Amount of Standard Added (µg/ml)	Total concentration (µg/ml)	Mean peak area	% Recovery	% RSD
80	16	20	36	5605856	101.22	0.35
100	20	20	40	5621363	101.5	0.45
120	24	20	44	5554350	100.29	0.34

DISCUSSION:

1. % Recovery of HCT was found to be in the range of 100.47%- 101.35% and %RSD was found to be between 0.55%- 1.02%.

2. % Recovery of AMLwas found to be in the range of 100.26%- 100.98% and %RSD was found to be between 0.75%- 1.09%.

3. % Recovery of TEL was found to be in the range of 100.09%- 101.5% and %RSD was found to be between 0.34%- 0.45%.

4. From the above observation it was concluded that the method is accurate for the simultaneous determination of HCT, AML & TEL in a tablet dosage form.

2. Precision: The results of precision method were evaluated by carrying out six independent test samples of HTZ, AML and TLM. The percentage of RSD of six sample peak area values were calculated. The RSD values of intra-day and inter-day studies for HTZ, AML and TLM confirming good precision of the optimized method.

a) Method Precision: The method precision of the method was established by carrying out the analysis of analyte (n=6) using the proposed method. The low value of relative standard deviation showed that the method was precise. The results obtained were presented in **Table 7**.

TABLE 7: SYSTEM PRECISION FOR ASSAY OF HCT, AML AND TEL

Standard Solution			
	HCT	**AML**	**TEL**
Tailing Factor	1.235	1.362	1.529
Theoretical Plates	2503	2193	4056
Mean peak area (n = 6)	344646	341767	5520983
% RSD	1.28	0.87	0.2

b) System precision: The system precision of the method was established by six replicate injections of the standard solution containing both the analytes of interest. The percentage RSD was calculated and presented in **Table 8, 9**.

Results for Intra-day Precision:

TABLE 8: METHOD PRECISION FOR ASSAY METHOD OF HCT, AML AND TEL

Sample No.	Area		
	HCT	AML	TEL
1	345197	340568	5520615
2	339985	339658	5520395
3	348756	345164	5521940
4	344646	341766	5520983
5	347856	348756	5513589
6	345685	346859	5568496
Mean	345354	343795	5527670
% RSD	0.89	1.07	0.37

Results for Intra-day Precision:

TABLE 9: METHOD PRECISION FOR ASSAY METHOD OF HCT, AML AND TEL

Sample No.	Area		
	HCT	AML	TEL
1	340568	345197	5530615
2	339658	339985	5525395
3	345164	348756	5521940
4	341766	344646	5521983
5	348756	347856	5513589
6	346859	345685	5568496
Mean	343795	345354	5530336
% RSD	1.07	0.89	0.35

DISCUSSION: The % RSD values indicate an acceptable level of precision of the analytical system for the assay of HCT, AML and TEL. The % RSD values on lower side indicate that there exists only minimum variation in the results and thus the system precision and method precision is validated.

3. Linearity: Linearity studies were performed at six different concentrations. The linearity data was calculated on individual drug basis and checked for the R^2 value.

TABLE 10: LINEARITY DATA OF HCT FOR ASSAY METHOD

HCT	Area
6	170070
12	340796
18	516985
24	694404
30	885894
36	1050420

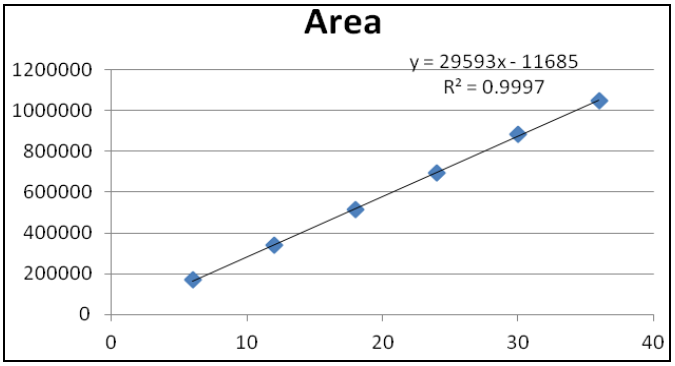

FIG. 4: LINEARITY CURVE FOR HCT

TABLE 11: LINEARITY DATA OF AML FOR ASSAY METHOD

AML	Area
2.5	274151
5	426693
7.5	580884
10	751419
12.5	925627
15	1080752

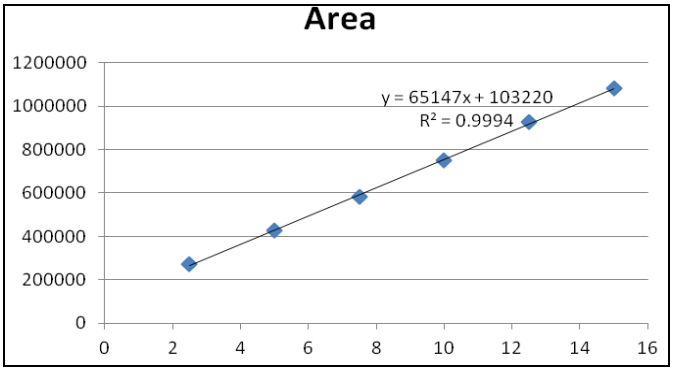

FIG. 5: LINEARITY CURVE FOR AML

TABLE 12: LINEARITY DATA OF TEL FOR ASSAY METHOD

TEL	Area
20	2653556
40	5554518
60	8570562
80	11589481
100	14769199
120	17521336

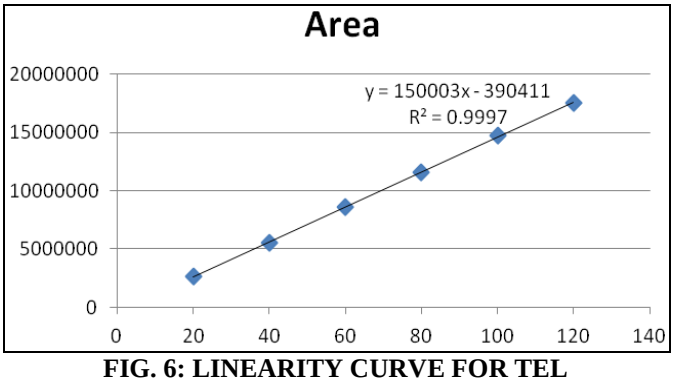

FIG. 6: LINEARITY CURVE FOR TEL

Discussion: Linearity was established for HCT within 6-36µg/ml, for AML within 2.5-15µg/ml and for TEL within 20-120µg/ml. Graphs of Concentration v/s Peak Area were plot. Regression equation for HCT, AML and TEL was obtained. Regression co-efficient for linearity of HCT is 0.9997, for AML 0.9994 and that for TEL is 0.9997.

4. Ruggedness: Ruggedness of the method was verified by analyzing six samples of the same concentration used for method precision as per proposed method by different analysts.

TABLE 13: EFFECT OF CHANGE IN ANALYST FOR HCT, AML AND TEL

System Suitability Parameter	Observation			Limits
	Drug	Analyst 1	Analyst 2	
The % RSD of peak area response	HCT	1.24	1.28	NMT 2%
	AML	0.87	1.00	
	TEL	0.10	0.20	
Theoretical plates	HCT	2612	2598	NLT 2000
	AML	2198	2535	
	TEL	4028	4056	
Tailing Factor	HCT	1.235	1.258	NMT 2
	AML	1.362	1.351	
	TEL	1.529	1.589	
Retention Time (min.)	HCT	2.983	2.953	
	AML	5.055	5.120	
	TEL	8.133	8.187	

Discussion: The % RSD values for intermediate precision were found to be within the specified limit. This indicated that the method is rugged and can be used to analyze the formulations containing HCT, AML and TEL effectively in any given condition.

5. Robustness: Robustness of the method was evaluated by changing the flow rate by ± 10 %, by changing the wavelength by ± 5 nm and by changing the pH of buffer by ± 0.2 units.

a. Effect of Variation in flow rate of mobile phase by ± 10%:

TABLE 14: EFFECT OF VARIATION IN FLOW RATE OF MOBILE PHASE BY ± 10%

Sr. No.	System Suitability Parameter	Observations				Limits
		Drug	0 % (1ml/min)	- 10% (0.9ml/min)	+ 10% (1.1ml/min)	
1.	The % RSD of peak area response	HCT	1.24	1.32	1.18	NMT 2%
		AML	0.87	0.98	0.62	
		TEL	0.10	0.48	0.35	
2.	Theoretical plates	HCT	2503	2497	2598	NLT 2000
		AML	2193	2048	2281	
		TEL	4056	3982	4158	
3.	Tailing Factor	HCT	1.235	1.352	1.206	NMT 2
		AML	1.362	1.405	1.318	
		TEL	1.529	1.586	1.494	
4.	Retention Time (min.)	HCT	2.983	3.055	2.942	
		AML	5.255	5.324	5.165	
		TEL	8.133	8.251	8.051	

Discussion: Small but deliberate variations were made in the method parameters and it was found that % RSD values for all the variations were in acceptable limits. This indicated that the method is robust and that it can be used within small variations of flow rate, pH and wavelengths without having a major effect on the results of % assay values.

b. Effect of Change in wavelength of analysis by ± 5 nm:

TABLE 15: EFFECT OF CHANGE IN WAVELENGTH OF ANALYSIS BY ± 5 nm

Sr. No.	System Suitability Parameter	Observations				Limits
		Drug	0 % (239 nm)	+ 5nm 244 nm	- 5nm 234 nm	
1.	The % RSD of peak area response	HCT	1.24	1.35	1.12	NMT 2%
		AML	0.87	0.96	0.74	
		TEL	0.10	0.35	0.24	
2.	Theoretical plates	HCT	2503	2492	2540	NLT 2000
		AML	2193	2165	2203	
		TEL	4056	4132	4068	
3.	Tailing Factor	HCT	1.235	1.224	1.315	NMT 2
		AML	1.362	1.342	1.458	
		TEL	1.529	1.596	1.465	
4.	Retention Time (min.)	HCT	2.983	2.895	2.996	
		AML	5.055	5.124	4.985	
		TEL	8.133	8.025	8.154	

c. Effect of Change in mobile phase pH by ± 0.2 units:

TABLE 16: EFFECT OF CHANGE IN MOBILE PHASE pH BY ± 0.2 UNITS

Sr. No.	System Suitability Parameter	Observations				Limits
		Drug	0 % (pH 3.2)	- 10% (pH 3.0)	+ 10% (pH 3.4)	
1.	The % RSD of peak area response	HCT	1.24	1.12	1.05	NMT 2%
		AML	0.87	0.95	0.76	
		TEL	0.10	0.32	0.25	
2.	Theoretical plates	HCT	2503	2705	2698	NLT 2000
		AML	2193	2085	2157	
		TEL	4056	4125	4215	
3.	Tailing Factor	HCT	1.235	1.325	1.250	NMT 2
		AML	1.362	1.354	1.402	
		TEL	1.529	1.514	1.560	
4.	Retention Time (min.)	HCT	2.983	2.852	2.998	
		AML	5.055	5.114	5.087	
		TEL	8.133	8.214	8.098	

6. LOD (Detection limit): The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. For assay purpose limit of detection was not considered as the validation parameter as per ICH guideline

7. LOQ (Quantitation limit): The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. Therefore this is not considered as the validation parameter for assay purpose.

8. Specificity: To ensure the absence of interference from blank in Hydrochlorothiazide, Amlodipine besylate and Telmisartan drug product. One blank solution of diluent was injected and Hydrochlorothiazide, Amlodipine besylate and Telmisartan were injected individually.
Results are shown in **Fig. 1, 2** and **3**.

Discussion: There was no interference from blank at the retention time of Hydrochlorothiazide, Amlodipine besylate and Telmisartan.

CONCLUSION: The method was developed and validated which concluded that the method is linear, simple, accurate, precise, rugged. Thus, the proposed method can be effectively applied for analysis of HCT, AML and TEL in bulk dosage forms as well as in combined tablet dosage form.

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