Original Article

Estimation and Validation of A Polypill Containing Amlodipine Besylate, Hydrochlorothiazide and Telmisartan by RP-HPLC

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ABSTRACT

Objective: A new, simple, accurate RP-HPLC method was developed for the simultaneous estimation of drugs in a polypill containing amlodipinebesylate, hydrochlorothiazide and telmisartan by RP-HPLC.
Method: Good chromatographic separation was accomplished in isocratic mode using mixed phosphate buffer: acetonitrile (55:45) as mobile phase, and Kromasil (250 mm × 4.6 mm i.d, 5 µm particle size) column as stationary phase havinga flow rate of 1.0 mL/min at detection wavelength of 254 nm.
Results: The time of retention for hydrochlorothiazide, amlodipine and telmisartan was established at 3.2 min, 4.5 min and 5.7 min respectively. This method was validated as per ICH guidelines.[21,22]
Conclusion: This low cost method was proved to be successful when applied to pharmaceutical dosage forms without any interference in the chromatogram.

Keywords: Polypill, RP-HPLC, Amlodipine Besylate, Hydrochlorothiazide, Telmisartan.

INTRODUCTION

Polypill, a term proposed by Wald and Law, is a fixed dose combination of three or more drugs in a single pill for the prevention of a cardiovascular diseases with the aim of reducing the number of tablets or capsules.[1,2] Developing an analytical methodology for simultaneous estimation of individual drugs in a polypill is challenging due to the high number of components present in the dosage forms.[3]

Amlodipine besylate, chemically is (*RS*) - 3 – ethyl - 5 - methyl – 2 - [(2 - aminoethoxy) methyl] – 4 - (2 - chlorophenyl) – 6 – methyl - 1, 4 – dihydropyridine - 3, 5 – dicarboxylate, and is a potent dihydropyridine calcium

antagonist that prevents the influx of Ca^{2+} into vascular smooth muscle and cardiac muscle, thereby causing relaxation of the muscles lining the arteries in the body, hence reducing blood pressure and preventing coronary spasm. Hydrochlorothiazide, chemically designated as 6-chloro-1, 1-dioxo-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7-sulfonamide, is a thiazide diuretic, which blocks facilitated Na/Cl co-transport in the early distal tubule, decreasing the amount of fluid in the body by increasing the amount of salt and water lost in the urine. Telmisartan is chemically identified as2 - (4 - {[4 – methyl – 6 - (1 – methyl – 6 - (1- methyl - 1*H* - 1, 3 – benzodiazol - 2yl) – 2 – propyl - 1*H* - 1, 3 – benzodiazol – 1 - yl] methyl} phenyl) benzoic acid, is angiotensin receptor blocker, dilating the peripheral blood vessels thereby, decreasing the blood pressure.[4]

Fixed dose combination containing amlodipine besylate 5 mg, hydrochlorothiazide 12.5 mg, telmisartan 40 mg in tablet form are available in the market. All the three drugs' monographs are available in USP, BP[5,6] while amlodipine besylate, hydrochlorothiazide monographs are available in Indian pharmacopoeia (IP).[7]

Literature survey showed that numerous methods were described for estimation of individual drugs - amlodipine besylate, hydrochlorothiazide and telmisartan and in combination with additional drugs,[9-20] Only two reports are available for the estimation of amlodipine, telmisartan hydrochlorothiazide,[8, 20]in which both of them aregradient mode methods, and involve more time. The buffer pH used was highly acidic which may result in damage to the column. Nalwade and group used UPLC system which is expensive and is not available in small laboratories and educational institutions.[8] Therefore the current study aimed to develop a simple, inexpensive and accurate method for the simultaneous determination of amlodipine besylate, telmisartan and hydrochlorothiazide by RP-HPLC in formulation. In this paper, we have compared all our results with ICH guidelines.[21,22]

EXPERIMENTAL

Chemicals

Pure drug samples of amlodipine besylate, hydrochlorothiazide, telmisartan was obtained as gift sample from Aurobindo Drugs, Hyderabad. HPLC grade acetonitrile was acquired from Standard Reagents, Hyderabad, India. HPLC grade water was acquired from Merck. Potassium dihydrogen phosphate and dipotassium hydrogen phosphate of analytical gradewas procured from Qualigens Fine chemicals, Mumbai. Tablets of the polypill with brand name Telma Am H manufactured by Glenmark Pharmaceuticals Ltd, Mumbai was procured from a local pharmacy store.

Equipment

The Liquid chromatographic system used was Shimadzu (SPD 10ATVP) having a single wavelength detector. The output signal was processed using Spinchrom software. The UV Spectrophotometer was from Analytical Technologies Ltd.

Chromatographic conditions

The chromatographic separation was carried out on a C18-Kromasil column (250 mm ×4.6 mm) with 5 µm particles. The mobile phase consisted of a mixture of mixed phosphate buffer: acetonitrile (55: 45). Mixed phosphate buffer was prepared by dissolving 0.3 gm of dipotassium hydrogen orthophosphate and 1.625 gm of potassium dihydrogen orthophosphate in 550 mL of HPLC grade water. The prepared buffer was filtered using 0.45 µm membrane filter. Flow rate of the mobile phase was maintained at 1.0 mL/min. The volume of injection me was 20 µL.

Preparation of various buffers used in trials

Mixed Phosphate buffer (pH 3)

Mixed phosphate buffer (pH 3) was prepared by dissolving 0.3 gm of dipotassium hydrogen orthophosphate and 1.625 gm of potassium dihydrogen orthophosphate in 550 ml of HPLC grade water and the pH was adjusted to 3 ± 0.2 with orthophosphoric acid. Then the prepared buffer was filtered through 0.45 µm membrane filter.

Ammonium phosphate buffer (pH 6)

Ammonium phosphate buffer was prepared by dissolving 11 gm of ammonium dihydrogen orthophosphate in 500 ml of HPLC grade water and the pH was adjusted to 6 ± 0.2 by using orthophosphoric acid. Thereafter prepared buffer was filtered through 0.45 µm membrane filter.

Dipotassium hydrogen orthophosphate buffer (pH 6.5)

Dipotassium hydrogen ortho-phosphate buffer with pH 6.5 was prepared by dissolving 3.484 % (w/v) of dipotassium hydrogen orthophosphate in HPLC grade water and the pH of the solution was adjusted to 6.5 ± 0.2 with phosphoric acid and then the prepared buffer was filtered through 0.45 µm membrane filter.

Triethylamine phosphate buffer (pH 3)

Triethylamine phosphate buffer was prepared by dissolving0.1% (w/v) Orthophosphoric acid solution in HPLC grade water and the pH of the buffer was adjusted to 3 ± 0.2 using triethylamine. The prepared buffer was filtered through 0.45 µm membrane filter.

Mixed Phosphate buffer

Mixed phosphate buffer was prepared by dissolving 0.3 gm of dipotassium hydrogen orthophosphate and 1.625 gm of potassium dihydrogen orthophosphate in 550 ml of HPLC grade water and the prepared buffer was filtered through 0.45 µm membrane filter.

Preparation of Standard solutions

Stock solution of standard drugs was prepared by taking telmisartan- 80 mg, hydroclorothiazide-25 mg and amlodipine besylate-10 mg into 50 mL standard flask. 25 mL of the mobile phase was added and sonicated for 5 minutes to dissolve the drugs. The volume was made upto the mark with the mobile phase. From the prepared standard stock solution, 1 mL was taken into a 10 mL volumetric flask and the volume was made upto 10 mL with the mobile phase to obtain a concentration of 50µg/mL, 30µg/mL, 160µg/mL for hydro-chlorothiazide, amlodipine, telmisartan respectively.

Preparation of Sample solutions

Twenty tablets were taken, weighed and powdered. The resulting powder having weight equivalent to hydrochlorothiazide-25 mg, amlodipine besylate -10 mg and telmisartan-80 mg of was weighed accurately and taken into a 50 mL volumetric flask. To it, 25 mL of the prepared mobile phase was supplemented and sonicated for about 5 minutes to dissolve the drugs thoroughly. The volume was made upto the mark with the mobile phase. The resulting solution was filtered using a 0.45 µm membrane filter to prepare a stock solution of the tablet sample. From the above prepared test stock solution, 1 mL of the solution was taken into a 10 mL volumetric flask and the volume was made upto the mark with the mobile phase. The concentration of hydrochlorothiazide, amlodipine, and telmisartan in the solution was 50µg/mL, 30 µg/mL, 160 µg/mL respectively.

RESULTS AND DISCUSSION

Chromatographic Method Development

The standard drugs were run using different mobile phases at different pH and using organic modifiers such as acetonitrile and water in the mobile phase. The peak shapes was found to be symmetrical in adopted chromatographic conditions (Trial 9 and Table 1), though the tailing factor for telmisartan was found to be slightly above 2. Further trials were conducted to reduce tailing by the modification of pH which resulted in the splitting of the peaks or decrease in the resolution between the two drugs. Modification of the organic phase was also attempted which resulted in merging of peaks or no changes in the tailing of the drug.

Selection of Wavelength

The individual standard solutions of the chosen drugs at concentration of 100 µg/mL in the chosen mobile phase were scanned on UV Spectrophotometer from 200 to 400 nm are shown in Fig. 1. Detection was carried out at 254 nm on the basis of higher response of the drug.

Optimized Conditions

After thorough optimization (Table 1), the chromatographic conditions with good shapes were achieved onC18 `Kromasil column (250 mm × 4.6 mm) with 5 µm particles. The mobile phase consisted of mixed phosphate buffer: acetonitrile (55: 45) maintained at a flow rate of 1mL/ min. The detection was monitored at a wavelength of 254 nm. The injection volume given was 20 µL. The concentration of hydrochlorothiazide, amlodipine, telmisartan in the solution was 50 µg/mL, 30 µg/mL, 160 µg/mL respectively in both the standard solution and the sample solution. The retention times for the standard drugs (Fig. 2) were found to be 3.233`, 4.453, 5.873 minutes for hydrochlorothiazide, amlodipine and telmisartan respectively.

The above proposed method was applied for the analysis of tablet sample of Telma Am H (Fig. 3) and the outcome of the assay was obtained within the specification limit. The % Assay of amlodipine besylate, hydrochlorothiazide, telmisartan was 101.23, 101.74 and 100.78 % repectively. See fig. 2, 3 & table no. 1.

Method Validation

System suitability

To determine the satisfactory resolution and repeatability of the above proposed method, system suitability tests were performed[21,22] and results were presented in table 2. The parameters such as retention time, no. of theoretical plates, asymmetry factor, resolution were investigated (Table 2) by injecting standard solutions of the drugs six times. The % RSD for the retention time of amlodipine, hydrochlorothiazide, telmisartan was found to be less than 2%. The above mentioned parameters of amlodipine, hydro-chlorothiazide and telmisartan were well within the limits.

Specificity

The peaks of the drugs was evaluated by comparison of retention time of standard drugs with the sample[21,22]. Good correlation was observed between the retention time of standard (Fig.2) and sample (Fig.3) It was observed from that the diluent or the excipient peaks did not interfere with the hydrochlorothiazide, amlodipine, telmisartan peaks.

Linearity

Solutions required for the assessment of linearity were prepared from the stock solutions at different concentration levels

ranging from 50 to 150 % of the analyte concentrations. A graph was plotted between the drug concentration on the x axis and peak area on y axis[21,22] (Fig. 4 to 6). The calibration curves were observed to be linear in the range of 12 to 28 µg/mL for amlodipine besylate, 30 to 70 µg/mL for hydrochlorothiazide and 96 to 224 µg/mL for telmisartan with linear correlation coefficient of more than 0.99 for all the three drugs (Table 3).

Precision

The precision at 100 % concentration of the assay method was assessed by carrying out six independent assays[21,22] of hydrochlorothiazide, amlodipine besylate, telmisartan with the reference standard of the same drugs. The results were given Table 4 and Table 5, found to be within the given limits.

Accuracy

The accuracy of the method was estimated in triplicates by performing recovery studies at three diverse concentration levels of 80%, 100 %, 120%. Measured amounts of standard drug concentrations was added to the sample.[21,22]The % mean recovery of amlodipine, hydrochlorothiazide, telmisartan was found to be 100.72, 100.52, 101.59 respectively (Table 6) and the results were found to be satisfactory.

Robustness

The robustness of the developed method, small deliberate variations in the optimized parameters were carried out .[21,22] The consequence of changes in flow rate and wavelength of detection on retention time and tailing factor were examined. The method was examined to be unaffected by the small changes such as variation of ± 0.2 mL/min in flowrate of mobile phase andvariation in ± 2 nm in detection wavelength (Table 7).

Limit of Detection (LOD)

The limit of detection for the drugs amlodipine besylate, hydrochlorothiazide and telmisartan can be calculated from the formula,

$$LOD = 3.3\left(\frac{SD}{S}\right)$$

SD- Standard Deviation of peak area, *S*- Slope.[21,22]

For this method, the LOD value was calculated to be 0.89 µg/mL for amlodipine besylate, 2.046µg/mL for hydro chlorothiazide and 10.76µg/mL for telmisartan.

Limit of Quantification (LOQ)

The limit of quantitation for the drugs amlodipine besylate, hydro-chlorothiazide, telmisartan can be calculated from the formula,

$$LOD = 10\left(\frac{SD}{S}\right)$$

Where, *SD*- Standard Deviation of peak area,*S*- Slope.[21,22]

For this method, the LOQ value was calculated to be 2.72 µg/mL for amlodipine besylate, 6.20µg/mL for hydrochlorothiazide and 32.60µg/mL for telmisartan.

CONCLUSION

A new, simple, accurate RP-HPLC method was successfully developed for the quantitative estimation of amlodipine besylate, hydrochlorothiazide, telmisartan in pharmaceutical tablet dosage form by an isocratic mode. The method is specific as no interference is observed from excipient of diluent. The method is simple with well separated peaks within a total run time of 8 minutes. The instrumentation used for this method development was a regularly available HPLC system which is economical

when compared to the gradient instrumentation or a UPLC system. All the parameters in this study were evaluated to be well within the limits according to ICH guidelines. This method can be applied to repetitive analysis in various quality control laboratories and educational institutions.

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Table 1: Overview of chromatographic conditions for various trials

Condition	Trial-1	Trial-2	Trial-3	Trial-4	Trial-5	Trial-6	Trial-7	Trial-8	Trial-9
C 18 Column (250 mm × 4.6 mm × 5 µm)	Hypersil						Inertsil		Kromasil
Mobile Phase	MeOH: Water (60:40)	MPB (pH 3): ACN (55:45)	MPB (pH 3): ACN (80:20)	APB (pH 6): ACN: MeOH (50:25:25)	DHPB (pH 6.5): ACN: Water (10:35:35)	TPB (pH 3): ACN: MeOH (50:25:25)	MPB – A MeOH-B	MPB : ACN (55:45)	MPB : ACN (55:45)
Elution mode	Isocratic						Gradient		Isocratic
Flow rate	1.0 mL/ min								
Detection wavelength	280 nm	280 nm	280 nm	240 nm	240 nm	240 nm	254 nm	254 nm	254 nm
Injection volume	20 µl								
Retention time (Min)	HCTZ- 3.69 TM- .91	HCTZ- 2.14	AM- 5.06 HCTZ- 8.59	AM- 2.16	HCTZ- 2.55 TM- 2.69	AM- 3.11 HCTZ - 3.25	HCTZ- 5.83 AM- 6.36 TM- 6.87	HCTZ- 3.66 AM- 6.59 TM– 9.12	HCTZ- 3.23 AM- 4.45 TM- 5.87

MeOH- Methanol, MPB- Mixed Phosphate buffer, APB- Ammonium phosphate buffer, DHPB- Dihydrogen phosphate buffer, TPB- Triethylamine phosphate buffer, ACN- Acetonitrile, HCTZ- Hydrochlorothiazide, TM-Telmisartan, AM-Amlodipine

Table 2: System suitability parameters for hydrochlorothiazide, amlodipine besylate, telmisartan

System suitability parameters	Hydrochlorothiazide	Amlodipine besylate	Telmisartan
Retention time	3.253	4.453	5.810
Repeatability of Retention time; RSD % (n=5)	0.314	0.521	0.627
Resolution	-	4.760	4.059
Tailing factor	1.863	1.522	2.143
Theoretical plates	3470	3955	3640

0.99 for all the three drugs (Table 3).

Table 3: The linearity data for amlodipine besylate, hydrochlorothiazide and telmisartan

Drug	Concentration range	Correlation coefficient	Slope	Intercept
Amlodipine Besylate	12 to 28 µg/mL	0.991	7.473	32.65
Hydrochlorothiazide	30 to 70 µg/mL	0.992	6.683	32.17
Telmisartan	96 to 224 µg/mL	0.991	13.03	242.7

Injection	Amlodipine		Hydrochlorothiazide		Telmisartan	
	RT	Area	RT	Area	RT	Area
1	4.507	178.727	3.273	372.099	5.793	2312.667
2	4.497	179.3	3.263	372.909	5.81	2392.035
3	4.453	178.69	3.253	369.428	5.81	2326.55
4	4.453	182.781	3.233	371.594	5.873	2372.046
5	4.477	183.898	3.263	381.487	5.807	2310.602
6	4.468	179.251	3.251	379.556	5.856	2325.532
Average	4.475	180.441	3.256	374.512	5.824	2339.9
SD	0.022	2.286	0.013	4.834	0.031	33.870
%RSD	0.502	1.267	0.423	1.290	0.546	1.447

Table 4: Results for precision of the sample

Injection	Amlodipine		Hydrochlorothiazide		Telmisartan	
	RT	Area	RT	Area	RT	Area
1	4.507	178.727	3.273	372.099	5.793	2312.667
2	4.497	179.3	3.263	372.909	5.81	2392.035
3	4.453	178.69	3.253	369.428	5.81	2326.55
4	4.453	182.781	3.233	371.594	5.873	2372.046
5	4.477	183.898	3.263	381.487	5.807	2310.602
6	4.468	179.251	3.251	379.556	5.856	2325.532
Average	4.475	180.441	3.256	374.512	5.824	2339.9
SD	0.022	2.286	0.013	4.834	0.031	33.870
%RSD	0.502	1.267	0.423	1.290	0.546	1.447

Table 5: Results for precision of the standard drugs

Injection	Amlodipine		Hydrochlorothiazide		Telmisartan	
	RT	Area	RT	Area	RT	Area
1	4.517	183.898	3.283	381.457	5.717	2333.602
2	4.507	179.727	3.273	372.099	5.793	2342.667
3	4.503	181.903	3.270	373.769	5.803	2442.609
4	4.497	179.301	3.263	372.909	5.810	2362.035
5	4.477	182.781	3.263	371.594	5.807	2372.046
6	4.453	178.690	2.253	369.428	5.810	2326.55
Average	4.492	181.049	3.267	373.542	5.790	2363.252
SD	0.023	2.107	0.010	4.144	0.036	42.488
%RSD	0.521	1.164	0.314	1.109	0.627	1.797

Table 6: Accuracy by recovery studies

Drugs	Conc.	Avg. area	Amount present	Amount added	Amount found	% recovery	Mean	% RSD
Amlodipine Besylate	80	265.12	12	16	28.02	100.06	100.72	0.909
	100	285.38	12	20	32.11	100.35		
	120	317.61	12	24	36.63	101.76		
Hydrochlorothiazide	80	513.42	30	40	70.11	100.16	100.52	0.903
	100	567.12	30	50	79.90	99.87		
	120	661.13	30	60	91.40	101.56		
Telmisartan	80	3277.8	96	128	228.53	102.02	101.59	0.488
	100	3724.9	96	160	260.36	101.70		
	120	4163.3	96	192	291.05	101.06		

Table 7: Results of robustness by variations in flow rate and wavelength

Para-meter	Value	Amlodipine		Hydro-chlorothiazide		Telmisartan	
		RT	TF	RT	TF	RT	TF
Flow-rate	0.8 mL/min	5.570	1.626	4.037	1.897	7.330	2.228
	1.0 mL/min	4.477	1.706	3.263	1.832	5.807	2.220
	1.2 mL/ min	3.663	1.693	2.650	1.791	4.813	2.075
Wave-length	252 nm	4.467	1.589	3.233	1.800	5.860	2.191
	254 nm	4.477	1.622	3.263	1.736	5.807	2.181
	256 nm	4.457	1.600	3.227	1.854	5.853	2.217

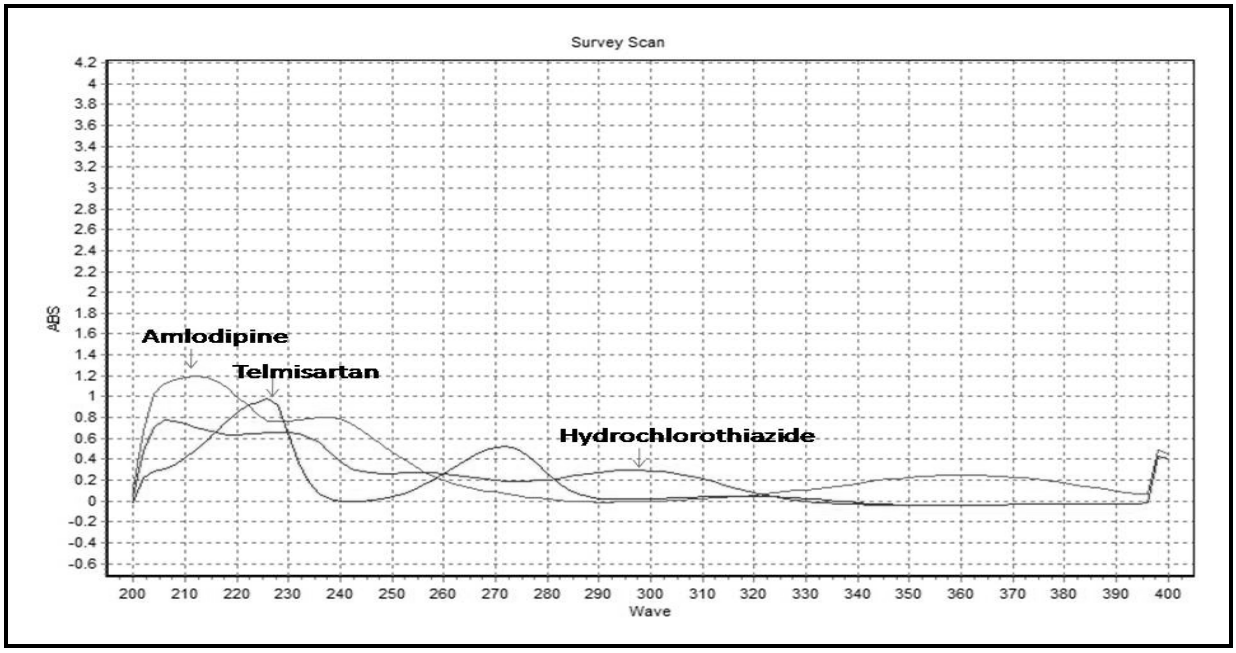

Figure 1. Selection of wavelength

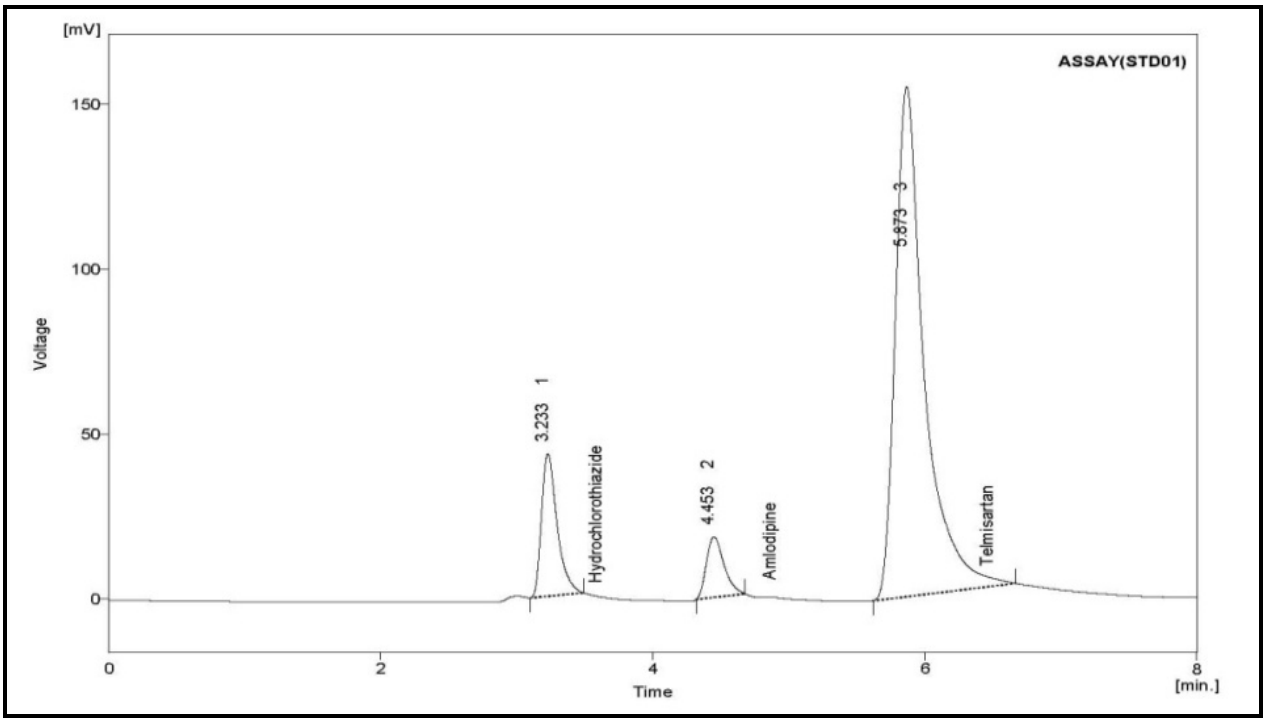

Figure 2. Chromatogram of standard solution for assay

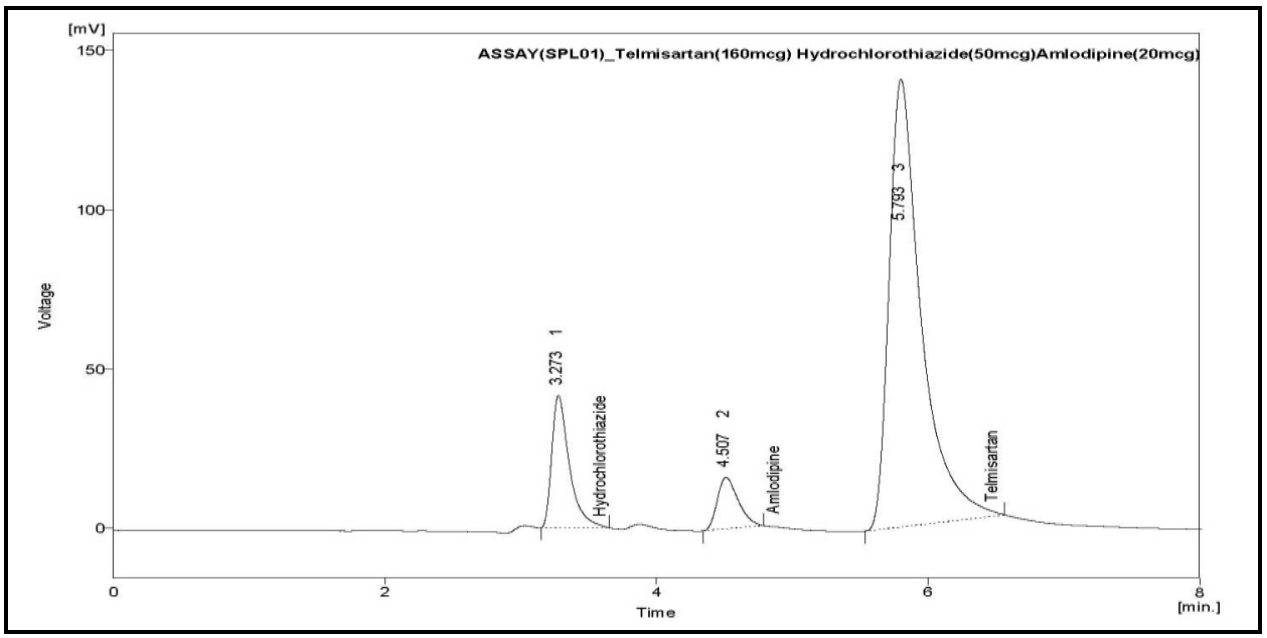

Figure 3. Chromatogram of sample solution for assay

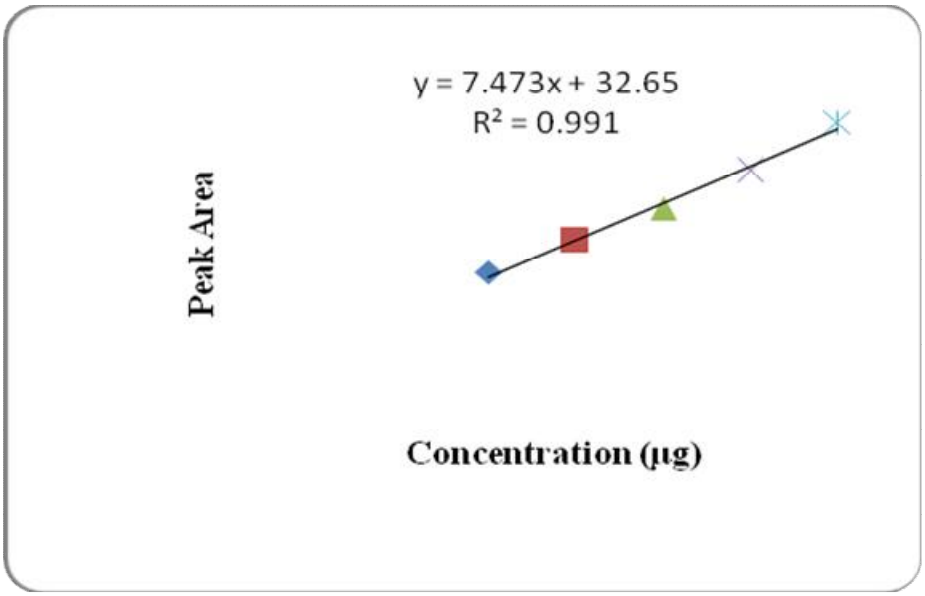

Figure 4. Calibration curve of amlodipine besylate

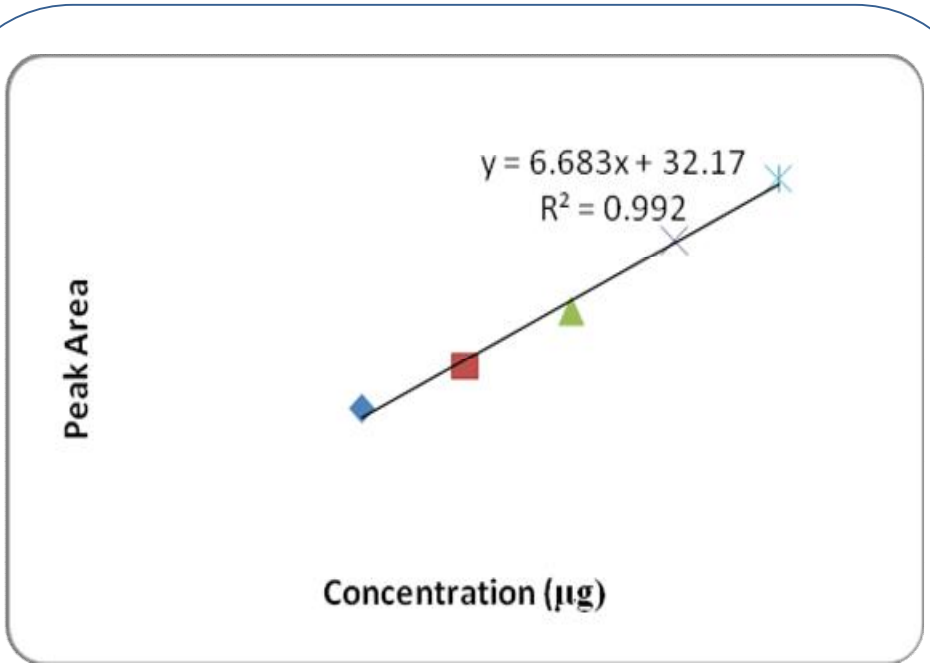

Figure 5. Calibration curve of hydrochlorothiazide

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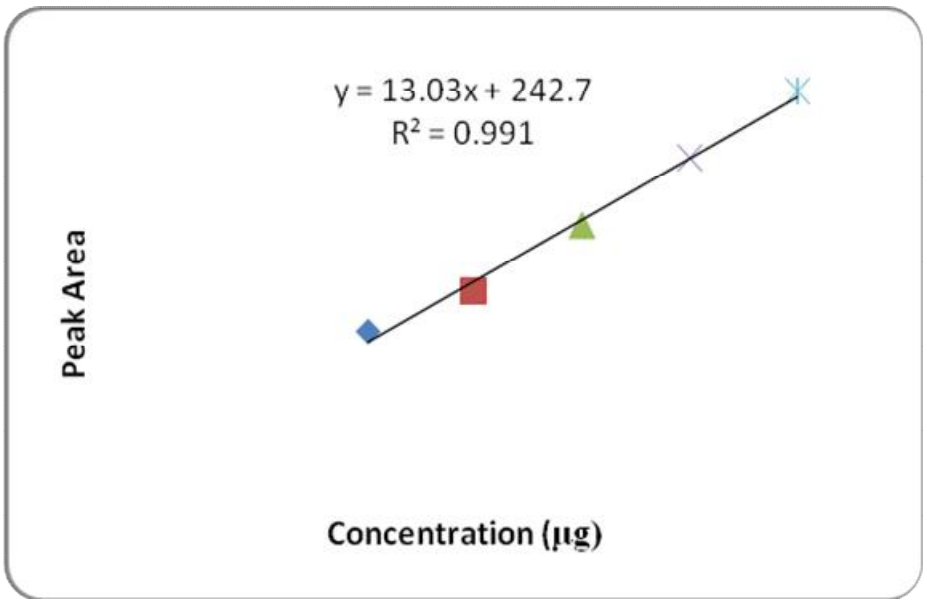

Figure 6. Release profile of KP through H2 formulations