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Analytical method development and validation of simultaneous determination of Amlodipine Besylate, Valsartan and Hydrochlorthiazide in oral dosage form (tablets) by RP-HPLC technique

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ABSTRACT

A simple, rapid, accurate, specific and sensitive RP-HPLC method has been developed and validated for the simultaneous estimation of Hydrochlorothiazide, Amlodipine Besylate and Valsartan in pharmaceutical oral dosage form (Tablets). The Chromatographic separation was performed on Hypersil BDS C_{18} column (250mm×4.6mm, particle size of 5µm) using a mobile phase of Water: Acetonitrile: Tri-Fluoroaetic acid in 55:45:0.1, at a flow rate of 1.0ml/min at an ambient temperature with detection wavelengths at 265, 237 and 265nm respectively for Hydrochlorothiazide, Amlodipine Besylate and Valsartan. The retention times was found to be 3.241, 6.836 and 9.634min respectively. The Linearity for Hydrochlorothiazide, Amlodipine Besylate and Valsartan was found in the concentration range of 6.25-18.75mcg/ml, 5-15mcg/ml and 80-240mcg/ml and correlation coefficient was found to be 0.9991, 0.9993 and 0.9988 respectively. The percentage purity of Hydrochlorothiazide, Amlodipine Besylate and Valsartan was found to be 99.52, 100.50 and 99.78% w/v respectively. The proposed method has been validated for accuracy, precision, linearity, range, system suitability and specificity were within the acceptance limit according to ICH guidelines and the developed method can be successfully employed for the routine quality analysis in pharmaceutical oral dosage form (tablets).

Key words: Hydrochlorothiazide, Amlodipine Besylate, Valsartan, RP-HPLC, Validation.

INTRODUCTION

Hydrochlorothiazide is chemically 6-chloro-1, 1-dioxo-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7-sulfonamide, its Molecular Weight is 297.74 g/mol., with an empirical Formula $C_7H_8ClN_3O_4S_2$. Hydrochlorothiazide is Anti-Hypertensive Agent, Diuretic and Sodium Chloride Symporter Inhibitor.

Amlodipine Besylate is chemically (RS)-3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5pyridine dicarboxylate benzene sulfonate, its Molecular Weight is 567.1g/mol., with an empirical Formula $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$. Amlodipine Besylate is an Anti-Hypertensive Agent, Vasodilator Agent and Calcium Channel Blocker.

Valsartan is chemically (*S*)-3-methyl-2-(*N*-{[2'-(2*H*-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl]methyl}pentanamido) butanoic acid, its Molecular Weight is 435.519 g/mol., with an empirical Formula $C_{24}H_{29}N_5O_3$. Valsartan is Angiotensin II receptor antagonist (more commonly called an ARB, or angiotensin receptor blocker), with particularly high affinity for the type I (AT_1) angiotensin receptor. By blocking the action of angiotensin, valsartan dilates blood vessels and reduces blood pressure. In the U.S., valsartan is indicated for treatment of high blood pressure, congestive heart failure (CHF), or post-myocardial infarction (MI).

Fig.No.1: Chemical structure of Hydrochlorothiazide

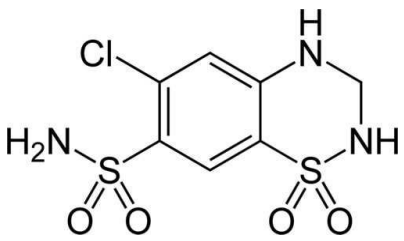


Fig.No.2: Chemical structure of Valsartan

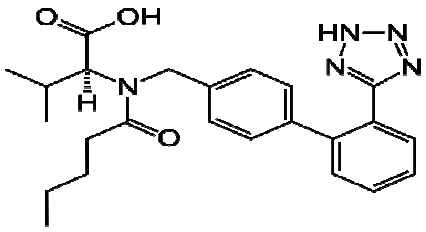
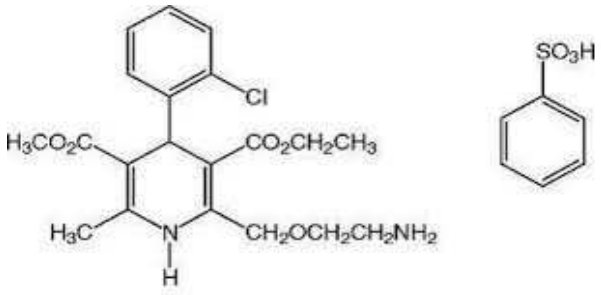


Fig.No.3: Chemical structure of Amlodipine Besylate



Literature survey reveals that few spectro-photometric methods [1-4], HPTLC methods [4,5] and HPLC methods [4-11] and has been reported for the estimation of Hydrochlorothiazide, Amlodipine Besylate and Valsartan. The aim of the present study is to develop a simple, precise and accurate Reversed-Phase HPLC method for the estimation of Hydrochlorothiazide, Amlodipine Besylate and Valsartan in pharmaceutical oral dosage form (tablets).

MATERIALS AND METHODS

Instrumental and Analytical Conditions:

Reagents and Chemicals:

The pharmaceutical drug samples Hydrochlorothiazide, Amlodipine Besylate and Valsartan were obtained as a gift from CTX Life Sciences Pvt. Ltd, Prudence Pharma chem., Par Drugs Ltd. (Chennai, Tamil Nadu) all the chemicals used were of HPLC grade such as Water which is processed from Milli-Q, NA in Sai Mirra Innopharm Pvt. Ltd and Acetonitrile obtained from ACROS organics which is of AR grade.

Equipment:

A LC-2010 AHT gradient system with LC solution software and a UV/Vis detector is the most sensitive and versatile dual wavelength absorbance detector was used. It was manufactured by the Shimadzu. Intelligent LC pump with sampler programmed at 10µlit capacity per injection was used.

Chromatographic conditions:

The column used was Hypersil BDS C_{18} Column (250×4.6mm, 5µm particle size) was used for analytical separation. The mobile phase consisted of Water, Acetonitrile and Tri-fluoro Acetic acid in the ratio of 55:45:0.1%v/v/v. The flow rate was adjusted to 1.0ml/min. The instrument was operated at ambient temperature. The UV detection was achieved at 265, 237 and 265nm respectively for Hydrochlorothiazide, Amlodipine Besylate and Valsartan. The injection volume was 10µlit capacity

Preparation of Analytical Solutions:

Preparation of Mobile Phase:

Mix a mixture of 55ml of Water (HPLC grade), 45ml of Acetonitrile (HPLC grade) and 0.1ml of Tri-fluoro acetic acid (AR grade) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Diluent Preparation:

Mobile phase was used as the diluent.

Preparation of the individual Hydrochlorothiazide standard preparation:

Weigh accurately 25mg of Hydrochlorothiazide Working Reference Standard into a 100 ml volumetric flask. Dissolve and dilute to volume with mobile phase and mix. Dilute 5ml of this solution to 100ml with mobile phase.

Preparation of the individual Amlodipine Besylate standard preparation:
Weigh accurately 28mg Amlodipine Besylate Working Reference Standard into a 100 ml volumetric flask. Dissolve and dilute to volume with mobile phase and mix. Dilute 5ml of this solution to 100ml with mobile phase.

Preparation of the individual Valsartan standard preparation:
Weigh accurately 320mg of Valsartan Working Reference Standard into a 100 ml volumetric flask. Dissolve and dilute to volume with mobile phase and mix. Dilute 5ml of this solution to 100ml with mobile phase.

Preparation of Hydrochlorothiazide, Amlodipine Besylate and Valsartan Standard and Sample solution (Marketed formulation):
Sample preparation: Weigh accurately 550 mg of the sample into a 100 ml volumetric flask. Add 50 ml of mobile phase and sonicate for 30 minutes. Cool and dilute to volume with mobile phase and mix well. Dilute 5ml of this solution to 100ml with mobile phase

Method Development and Validation of HPLC:
The suggested analytical method was validated according to ICH guidelines [12] with respect to certain parameters such as specificity, linearity, precision, accuracy and system suitability.

Specificity:
The retention time of major peak in the test solution corresponds to that of Hydrochlorothiazide, Amlodipine Besylate and Valsartan peaks in standard solution. No peaks are observed in the placebo solution, which indicates that, the placebo does not influence the assay analysis.

Linearity:
Express ability to obtain test results where directly proportional to the concentration of analyte in the sample. The linearity of the method was established by spiking a series of sample mixtures of Hydrochlorothiazide, Amlodipine Besylate and Valsartan of different concentration levels 6.25-18.75mcg/ml, 5-15mcg/ml and 80-240mcg/ml respectively are injected in to the HPLC system. Construct the calibration curves for the standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations linear regression was applied and slope-a, intercept-b, correlation coefficient-R^2 and standard error (Er) were determined.

Precision:
Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions. Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision /Ruggedness (It shows the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as analyst, instruments and dates). In order to determine precision, six independent sample solution preparations from a single lot of formulation 25.1mcg/ml Hydrochlorothiazide, 27.6mcg/ml Amlodipine Besylate and 320.9mcg/ml Valsartan was injected in to HPLC system, the retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained which are found to be within the specified limits.

Accuracy:
Accuracy was determined in terms of percentage recovery the accuracy study was performed for 80%, 100% and 120% for Hydrochlorothiazide, Amlodipine Besylate and Valsartan. Standard and sample solutions are injected in to the HPLC system in triplicate and the percentage recoveries of the Hydrochlorothiazide, Amlodipine Besylate and Valsartan were calculated. The area of each level was used for calculation of % recovery.

System suitability:
System suitability tests were carried out on freshly prepared standard stock solutions of Hydrochlorothiazide, Amlodipine Besylate and Valsartan. It was calculated by injecting standards in six replicates at regular interval and the values were recorded.

RESULTS AND DISCUSSION

The present investigation reported is a new RP-HPLC method development and validation of simultaneous determination of Hydrochlorothiazide, Amlodipine Besylate and Valsartan. The method developed was proceeding with wavelength selection. The optimized wavelength was a dual wavelength programming at 265 and 237nm.
In order to get the optimized RP-HPLC method various mobile phases and columns were used. From several trials final method is optimized with the following conditions:

The mobile phase consisting of water, acetonitrile and tri-fluoroaetic acid in 55:45:0.1% v/v/v. The flow rate was adjusted to 1.0ml/min and a load of nearly 10µlit. The column used is Hypersil BDS C_{18} column having dimensions of 250×4.6mm, having particle size of 5µm. The instrument was operated at ambient temperature. The UV detection was carried out at 265nm, 237nm and 265nm respectively for Hydrochlorothiazide, Amlodipine Besylate and Valsartan.

The specificity of the method was to determine whether there are any interference of any impurities (the presence of components may be unexpected to present) in retention time of analytical peak. The linearity was determined as linearity regression of the claimed analyte concentration of the range 6.25-18.75mcg/ml, 5-15mcg/ml and 80-240mcg/ml respectively. The calibration curve obtained by plotting the peak area versus concentration and presented in **Table-1** was linear and correlation coefficient was found to be 0.9991, 0.9993 and 0.9988 respectively. The precision of a method was ascertained from determinations of peak areas of six replicates of the solutions and the %RSD was found to be 0.40%, 0.69% and 0.36% respectively and presented in **Tables-2, 3, 4** respectively. The intermediate precision/ Ruggedness is presented in **Tables-5, 6, 7** respectively and the %RSD for 1st was found to be 0.51%, 0.77% and 0.32% and the %RSD for 2nd analyst was found to be 0.46%, 0.51% and 0.54% respectively.

The accuracy study was performed in 80%, 100% and 120%. The percentage recovery was determined for Hydrochlorothiazide, Amlodipine Besylate and Valsartan and was found to be 99.94%, 100.09% and 100.20% respectively and was presented in **Tables-8, 9, 10**. The system suitability parameters like Theoretical plates (N) were calculated and were found to be 5335, 5592 and 9902 respectively for Hydrochlorothiazide, Amlodipine Besylate and Valsartan and Tailing factor (T) was found to be 1.323, 1.819 and 1.196 respectively and were presented in **Table-11**. All the values obtained from the above parameters are within the limits so the proposed RP-HPLC method was accurate and precise.

Table 1: Linearity results for Hydrochlorothiazide, Amlodipine and Valsartan

Sample ID	Hydrochlorothiazide		Amlodipine		Valsartan	
	Concentration (mcg/ml)	Area	Concentration (mcg/ml)	Area	Concentration (mcg/ml)	Area
50% of operating concentration	6.25	184527	5	119135	80	1018728
80% of operating concentration	10	293816	8	191422	128	1613328
100% of operating concentration	12.5*	371308	10*	245099	160*	2075659
120% of operating concentration	15	453950	12	287377	192	2491250
150% of operating concentration	18.75	555579	15	361139	240	3050209
Correlation coefficient (r >0.995) Hydrochlorothiazide =0.999,Amlodipine= 0.999, Valsartan=0.998 *y-intercept (NLT ±2.0%) Hydrochlorothiazide = -0.88 %,Amlodipine= - 0.36%, Valsartan=- 0.20%*						

Fig. 4: Linearity plot for Hydrochlorothiazide

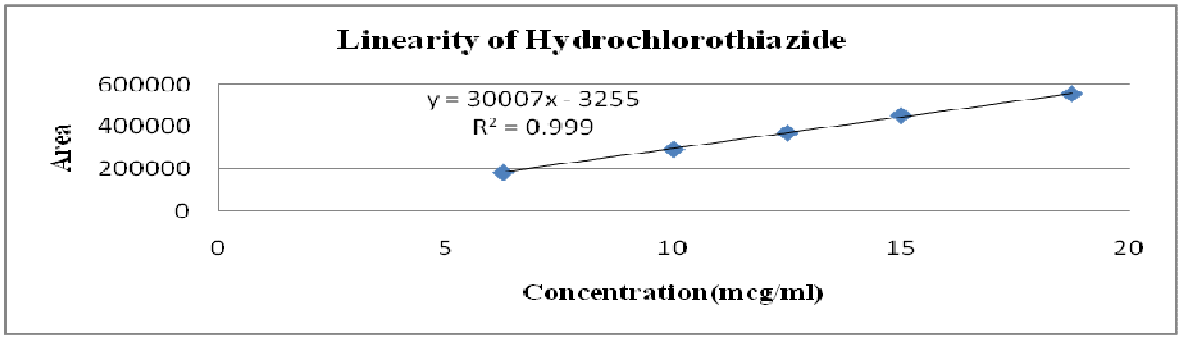


Fig. 5: Linearity plot for Amlodipine

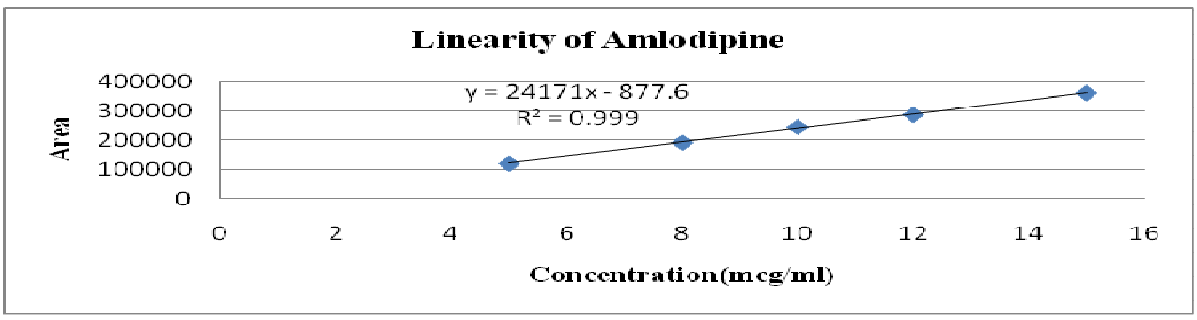


Fig. 6: Linearity plot for Valsartan

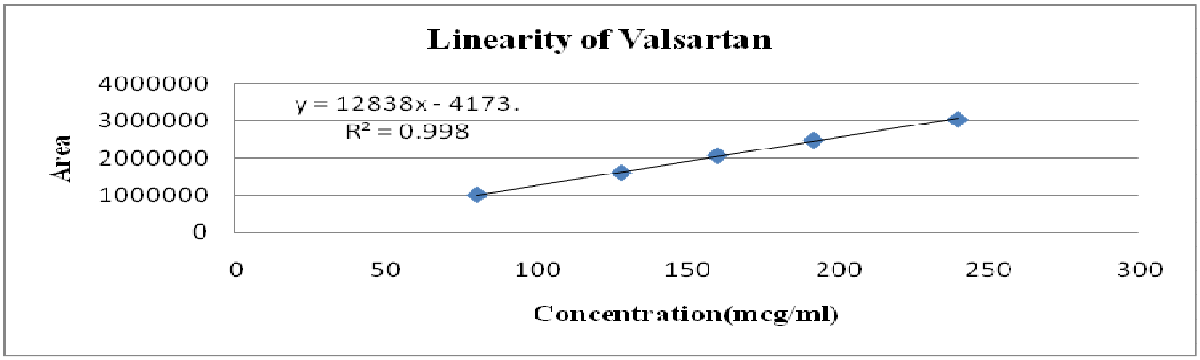


Table 2: Precision results for Hydrochlorothiazide

S. No.	Sample ID	Amount of preparation used mg	Peak response	Content (% of Label claim)
1	Sample-1	548.6	383345.5	12.46 mg (99.68%)
2	Sample-2	545.3	382899.5	12.52 mg (100.16%)
3	Sample-3	550.1	383025.0	12.41 mg (99.28%)
4	Sample-4	549.3	381172.0	12.37 mg (98.96%)
5	Sample-5	548.7	383224.0	12.45 mg (99.60%)
6	Sample-6	545.9	381440.0	12.46 mg (99.68%)
Mean				**12.44 mg (99.52%)**
RSD (NMT 2.0%)				**0.40%**

Table 3: Precision results for Amlodipine

S. No.	Sample ID	Amount of preparation used mg	Peak response	Content (% of Label claim)
1	Sample-1	548.6	250978.5	10.06 mg (100.60%)
2	Sample-2	545.3	250340.0	10.10 mg (101.0%)
3	Sample-3	550.1	250563.5	10.02 mg (100.20%)
4	Sample-4	549.3	247827.0	9.93 mg (99.30%)
5	Sample-5	548.7	250496.5	10.04 mg (100.40%)
6	Sample-6	545.9	251191.0	10.12 mg (101.2%)
Mean				**10.05 mg (100.50%)**
RSD (NMT 2.0%)				**0.69%**

Table 4: Precision results for Valsartan

S. No.	Sample ID	Amount of preparation used mg	Peak response	Content (% of Label claim)
1	Sample-1	548.6	2098495.0	159.38 mg (99.61%)
2	Sample-2	545.3	2096160.5	160.16 mg (100.1%)
3	Sample-3	550.1	2108340.0	159.69 mg (99.81%)
4	Sample-4	549.3	2091553.0	158.65 mg (99.16%)
5	Sample-5	548.7	2104596.5	159.81 mg (99.88%)
6	Sample-6	545.9	2098643.0	160.18 mg (100.11%)
Mean				**159.64 mg (99.78%)**
RSD (NMT 2.0%)				**0.36%**

Table 5: Ruggedness results for Hydrochlorothiazide

S. No.	**Day of analysis:22/03/2014**			**Day of analysis:24/03/2014**		
	Analyst : K.Karthik			**Analyst : N.Ravi Teja**		
	Chromatograph: QC/HPL/008			**Chromatograph: QC/HPL/007**		
	Amount of RS (mg) : 25.0mg			**Amount of RS (mg) : 25.2mg**		
	Peak response of Standard solution: 390567.5			**Peak response of Standard solution:379077.8**		
	Amount of preparation (mg)	**Peak response**	**Content (% of Label claim)**	**Amount of preparation (mg)**	**Peak response**	**Content (% of Label claim)**
1	545.8	389001.5	12.58mg(100.64%)	545.8	373243.0	12.53mg(100.24%)
2	550.8	387163.0	12.41mg(99.28%)	544.6	372994.5	12.54mg(100.32%)
3	545.3	387910.5	12.56mg(100.48%)	548.3	376891.5	12.59mg(100.72%)
4	545.5	387712.0	12.55mg(100.40%)	543.7	368930.0	12.43mg (99.44%)
5	542.3	386264.5	12.57mg(100.56%)	545.4	373653.0	12.55mg(100.40%)
6	543.3	385869.5	12.54mg(100.32%)	550.3	374808.0	12.48mg (99.84%)
	Mean		**12.53mg(100.24%)**	**Mean**		**12.52mg(100.16%)**
	RSD (NMT 2.0%)		**0.51%**	**RSD (NMT 2.0%)**		**0.46%**
Mean content: First analyst : 12.53mg ; Second analyst : 12.52mg						
RSD of content: First analyst : 0.51% ; Second analyst : 0.46%						

Table 6: Ruggedness results for Amlodipine

S. No.	Day of analysis:22/03/2014			Day of analysis:24/03/2014		
	Analyst : K.Karthik			Analyst : N.Ravi Teja		
	Chromatograph: QC/HPL/008			Chromatograph: QC/HPL/007		
	Amount of RS (mg) : 27.80mg			Amount of RS (mg) : 27.7mg		
	Peak response of Standard solution: 252944.5			Peak response of Standard solution:266327.2		
	Amount of preparation (mg)	Peak response	Content (% of Label claim)	Amount of preparation (mg)	Peak response	Content (% of Label claim)
1	545.8	250497.5	10.01mg(100.10%)	545.8	245598.5	10.04mg(100.40%)
2	550.8	252245.5	9.99mg(99.90%)	544.6	246052.0	10.08mg(100.80%)
3	545.3	248997.0	9.96mg(99.60%)	548.3	245495.5	9.99mg (99.90%)
4	545.5	249300.0	9.97mg(99.70%)	543.7	246983.0	10.13mg(101.30%)
5	542.3	244187.5	9.82mg(98.20%)	545.4	245179.5	10.03mg(100.30%)
6	543.3	250220.5	10.05mg(100.50%)	550.3	247241.5	10.02mg(100.20%)
	Mean		9.97mg (99.70%)	Mean		10.05mg(100.50%)
	RSD (NMT 2.0%)		0.77%	RSD (NMT 2.0%)		0.51%
Mean content: First analyst : 9.97mg ; Second analyst : 10.05mg						
RSD of content: First analyst : 0.77% ; Second analyst : 0.51%						

Table 7: Ruggedness results for Valsartan

S. No.	Day of analysis:22/03/2014			Day of analysis:24/03/2014		
	Analyst : K.Karthik			Analyst : N.Ravi Teja		
	Chromatograph: QC/HPL/008			Chromatograph: QC/HPL/007		
	Amount of RS (mg) : 320.5mg			Amount of RS (mg) : 319.5mg		
	Peak response of Standard solution:2134719.3			Peak response of Standard solution:2071774.5		
	Amount of preparation (mg)	Peak response	Content (% of Label claim)	Amount of preparation (mg)	Peak response	Content (% of Label claim)
1	545.8	2117990.5	160.82mg(100.51%)	545.8	373243.0	161.19mg(100.74%)
2	550.8	2119892.5	159.50mg(99.69%)	544.6	372994.5	160.64m(100.40%)
3	545.3	2108693.5	160.26mg(100.16%)	548.3	376891.5	159.21mg(99.51%)
4	545.5	2106535.0	160.04mg(100.03%)	543.7	368930.0	161.41mg(100.88%)
5	542.3	2103849.5	160.77mg(100.48%)	545.4	373653.0	160.96mg(100.60%)
6	543.3	2105236.0	160.58mg(100.36%)	550.3	374808.0	161.61mg(101.01%)
	Mean		160.33mg(100.21%)	Mean		160.84mg(100.53%)
	RSD (NMT 2.0%)		0.32%	RSD (NMT 2.0%)		0.54%
Mean content: First analyst : 160.33mg ; Second analyst : 160.84mg						
RSD of content: First analyst : 0.32% ; Second analyst : 0.54%						

Table 8: Recovery results for Hydrochlorothiazide

S.No	80% Recovery	100% Recovery	120% Recovery
1	99.90	100.16	99.93
2	100.20	99.92	99.80
3	99.90	100.32	99.40
Average	100.00%	100.13%	99.71%
RSD	0.17%	0.20%	0.28%

Table 9: Recovery results for Amlodipine

S.No	80% Recovery	100% Recovery	120% Recovery
1	100.25	100.10	99.84
2	99.88	99.90	99.75
3	100.37	100.40	100.33
Average	100.17%	100.13%	99.97%
RSD	0.25%	0.25%	0.31%

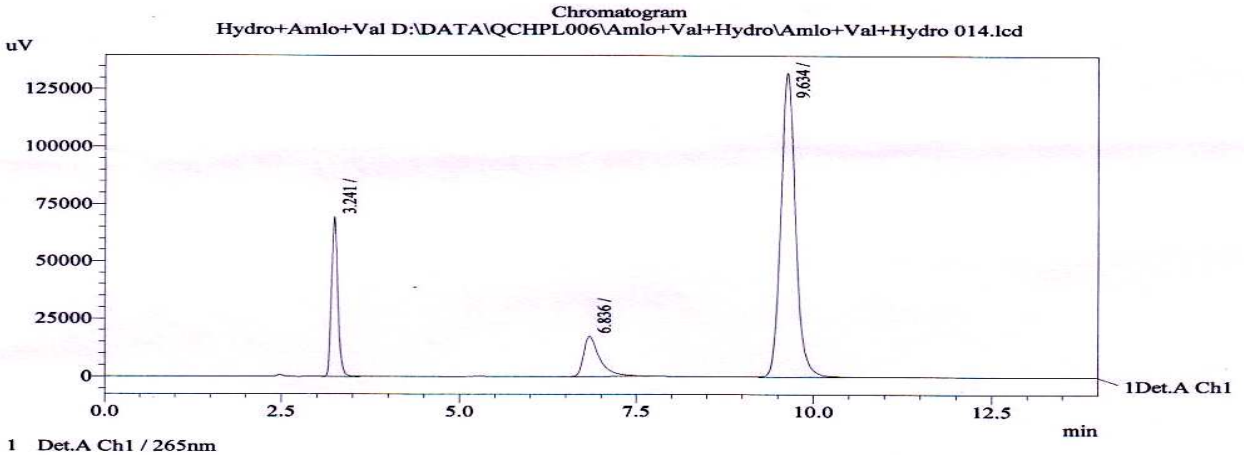
Table 10: Recovery results for Valsartan

S.No	80% Recovery	100% Recovery	120% Recovery
1	100.66	100.24	100.31
2	99.92	100.11	100.14
3	100.46	100.07	99.92
Average	100.35%	100.14%	100.12%
RSD	0.38%	0.09%	0.20%

Table 11: Showing System suitability parameters

S.No.	Parameters	Hydrochlorothiazide	Amlodipine	Valsartan
1	Average area	426233	272287	1901764
2	Retention Time (min)	3.241	6.836	9.634
3	Tailing factor	1.323	1.819	1.196
4	USP Plate Count	5335	5592	9902

Fig. 7: Standard Chromatogram of Hydrochlorothiazide, Amlodipine and Valsartan



PeakTable

Detector A Ch1 265nm

Peak#	Ret. Time	Area	Height	Theoretical Plate#	Tailing Factor	Resolution
1	3.241	426233	69470	5699.312	1.323	0.000
2	6.836	272287	17364	5054.134	1.819	12.922
3	9.634	1901764	132291	10586.535	1.196	7.372
Total		2600284	219126			

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for the determination of Hydrochlorothiazide, Amlodipine Besylate and Valsartan in their pharmaceutical oral dosage form (tablets). The method was validated for the parameters like specificity, linearity, accuracy, precision and system suitability values were found to be within the limits. The method has significant advantages in terms of shorter time of analysis, selectivity and accuracy than previously reported. The validation study indicates that the method can be considered suitable for carrying out quality control and routine determination of Hydrochlorothiazide, Amlodipine Besylate and Valsartan in their pharmaceutical oral dosage form (tablets).

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