Saminathan J. and Vetrichelvan T. / Vol 1 / Issue 1 / 2011/ 7-14.

International Journal of Pharmaceutical Research & Analysis

www.ijpra.com

METHOD DEVELOPMENT AND VALIDATION OF OLMESARTAN, AMLODIPINE AND HYDROCHLOROTHIAZIDE IN COMBINED TABLET DOSAGE FORM

J.Saminathan* and T.Vetrichelvan

*Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur, Tamil Nadu - 603 319.

ABSTRACT

A simple, accurate, precise, economical and reproducible method was developed for simultaneous estimation of olmesartan, amlodipine and hydrochlorothiazide in bulk and its dosage form. The excipients in the commercial tablet preparation did not interfere with the assay. Here we have developed a validated simultaneous estimation of olmesartan, amlodipine and hydrochlorothiazide in bulk and its tablet formulation. The stock solutions were prepared in methanol followed by the further required dilutions with Double distilled water. The λmax for olmesartan, amlodipine and hydrochlorothiazide were 256.5 nm, 239 nm and 271.5nm respectively. Linearity in concentration range of 4-24 µg/ mL, 1-10 µg/ mL and 2-20 µg/mL was shown respectively by the three drugs. The proposed method has estimated olmesartan 99.45±0.94%, amlodipine 98.95±0.32% and hydrochlorothiazide 100.46±0.68% in marketed tablets. The results of analysis have been validated statistically and also by recovery studies. Validation of the proposed methods was carried out for its accuracy, precision, and ruggedness according to ICH guidelines. Thus the present study gives an excellent method for the determination of all the three drugs in combined dosage formulation without their prior separation.

Keywords: Olmesartan medoxomil, Amlodipine besylate, Hydrochlorothiazide, Method validation, Simultaneous estimation, validation

INTRODUCTION

Olmat-AMH® is a combination of an angiotensin 2 receptor blocker, a dihydropyridine calcium channel blocker, and a thiazide diuretic indicated for the treatment of hypertension. It is not indicated for initial therapy. Olmesartanmedoxomil [1] (OLM), chemically it is 4-(1-Hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1H-tetazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl]-1H-imidazole-5-carboxylic acid (5-Methyl-2-oxo-1, 3-dioxol-4-yl) methyl ester. It works by blocking a substance in the body that causes blood vessels to tighten. As a result, olmesartan (fig.1) relaxes blood vessels. This lowers blood pressure and increases the supply of blood and oxygen to the heart. Amlodipine besylate [2] (AML) is Aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl 1,4dihydropyridine benzene sulfonate. It affects the movement of calcium into the cells of the heart and blood vessels. As a result, amlodipine (fig.2) relaxes blood vessels and increases the supply of blood and oxygen to the heart while reducing its workload. Hydrochlorothiazide [3] (HCTZ) is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide. It reduces the amount of water in the body by increasing the flow of urine, which helps lower the blood pressure. It is a fixed-dose triple combination has the potential to simplify dosing regimens, reduce pill burden, and reduce costs associated with copay[1]. These three medicines allow blood vessels to relax so that blood can flow more easily. The use of drug combinations is intended to increase the efficiency in chronic disease management due to synergic effect. olmesartan in combination with hydrochlorothiazide(fig.3) which increasing their hypotensive activity. Literature survey reveals that there is no reported method for the determination of olmesartan, amlodipine and hydrochlorthiazide. Some reported methods for the estimation of combinations using spectrophotometry [5-7], HPLC [8], HPTLC [9], LC-MS [10]. But no method has been found for the simultaneous estimation of Olmesartan, amlodipine and hydrochlorothiazide combinations.

Corresponding Author:- **J.Saminathan,** **Email:-** swamilingam@gmail.com

MATERIALS AND METHODS

Apparatus

SHIMADZU double beam UV-visible spectrophotometer (model 1700) with 1 cm matched quartz cuvettes were used for all absorbance measurements. Shimadzu AUX220 balance was used for weighing the samples**.** All chemicals and reagents used were of analytical grade. Milli-Q water and Whatmann filter paper (no.41) were used throughout the experimental work. OLM, AML and HCTZ Pure drugs (Accent Pharma. Pondy, INDIA), Methanol (LOBA, India Ltd) and double distilled water was used in the present study. The commercially available tablets Olmat-AMH® tablets containing a combination of OLM – 20 mg, AML- 5 mg and HCTZ-12.5 mg were procured from Micro Labs, Bangalore from local pharmacy,

Standard solution

Selection of common solvent

Methanol and double distilled water was selected as a solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

Determination of Absorption Maxima

By dilution of three standard drug solutions with methanol, solutions containing 10 µg ml^{-1} of OLM, 10 µg ml^{-1} of AMB and 10 µg ml^{-1} of HCTZ were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both all the drugs. OLM, AML and HCTZ showed absorbance maxima at 256.5 nm, 239 nm and 271.5 nm respectively. The overlain spectra showed λmax of all drugs (Fig. 4) below formula.

Three simultaneous equations [11-15] are framed based upon the fact that at λ_1, λ_2 and λ_3 the absorbance of the mixture is the sum of the individual absorbances of Olmesartan, Amlodipine and Hydrochlorothiazide.

The absorptivities of Amlodipine at 239 nm, 256.5 nm and 271.5 nm are ax_1, ax_2 and ax_3 respectively. The absorptivities of olmesartan at 239 nm, 256.5 nm and 271.5 nm are ay_1, ay_2 and ay_3 respectively.

The absorptivities of hydrochlorothiazide at 239 nm, 256.5nm and 271.5 are az_1, az_2 and az_3 respectively. The absorbance of sample solution at 239 nm, 256.5 nm and 271.5 are A_1, A_2 and A_3 respectively

At 239 nm

$$A_1 = 394C_x + 148.75\ C_y + 58.6\ C_z \quad (1)$$

At 256.5 nm

$$A_2 = 421.5\ C_x + 486.18\ C_y + 343.4\ C_z \quad (2)$$

At 271.5 nm

$$A_3 = 128.2\ C_x + 280.37\ C_y + 752.6\ C_z \quad (3)$$

Linearity study

40 mg OLM, 10 mg AML and 20 mg of HCTZof pure drug were prepared in 50 ml volumetric flask dissolved in methanol. For the linearity study, aliquots of the drug solutions were further diluted with double distilled water to get the final working standards of concentration ranges as OLM (4-24 µg/ml), AML (1-10µg/ml) and HCTZ (2-20µg/ml) respectively. The λ_{max} of olmesartan, amlodipine and hydrochlorothiazide were 256.5 nm, 239 nm and 271.5 nm respectively. The calibration graphs were plotted at the respective wavelength.

Analysis of sample solution

From the triturate of 20 tablets of Olmat-AMH, an amount equivalent to 20 mg of OLM, AML-5 mg and HCTZ-12.5 mg was weighed and dissolved in 25 ml of methanol and sonicated for 20 minutes. The solution was filtered in a 50 ml calibrated volumetric flask through whatmann filter paper no.41. The residue was washed three times with 5 ml methanol and then final volume of the solution was made up to the mark and then aliquots diluted with double distilled water containing OLM, 16 µg/ ml AML 4 µg/ ml and 10 µg/ ml HCTZ. The absorbance of standard and sample solutions were measured at 256.5 nm, 239 nm and 271.5 nm using solvent blank. The results were calculated shown in the Table 2.

Recovery studies

Accuracy of the method was checked by recovery studies, wherein sample was spiked with known quantity of standard drug of OLM, AML and HCTZ .The percentage recovery of OLM, AMLO and HCTZ was found to be 99.47%, 99.89% and 100.19% respectively. Results of recovery studies indicated that the method is rapid, accurate and reproducible shown in Table 3.

Method Validation

The method was validated according to ICH Q_2B guidelines for validation [16, 17] of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte. Results are shown in Table 2 to 8.

Linearity

Linearity was checked by diluting standard stock solution at six different concentrations. OLM was linear with the concentration range of 4 – 24 µg/ ml at 256.5 nm. AML showed the linearity in the range of 1 – 10 µg/ ml at 239 nm. HCTZ was linear in the concentration range of 2 – 20 µg/ ml at 271.5 nm. Calibration curves (fig.5, 6, 7) were plotted between concentration and absorbance of drugs.

Sensitivity

The limit of Detection (LOD) and limit of Quantitation (LOQ) parameters were calculated using the following equations; LOD = 3.3σ/ s and LOQ = 10σ/ s, where σ is standard deviation of y intercept of calibration curve (n = 6) and s is slope of regression equation. Optical validation parameters were calculated in the Table 1.

Accuracy

The accuracy of the method was based on the percentage recovery of the analyte that was added to weighed amounts at level I, II, III compared to the declared amounts. The results of determinations along with statistical assessment, including the mean, standard deviation, relative standard deviation (RSD %) are presented in the Table 3.

Precision

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability (Table 4) was performed by the analysis of formulation was repeated for six times with the same concentration. The amount of each drug present in the tablet formulation of OLM, AMLO and HCTZ was 100.15±0.53, 100.47±0.39 and 100.78±0.29. The % RSD is 0.642, 0.421 and 0.314 was calculated in the Table 4. The intermediate precision (Table 5) of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated and presented in the Table 6.

Ruggedness/Robustness

The ruggedness test is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. In present study, determination of OLM, AMB and HCT were carried out by using different instruments and different analysts shown in the Table 7 & 8.

RESULTS AND DISCUSSION

An attempt has been made to develop a fast, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of OLM, AML and HCTZ in their combined dosage form. In this method drug obeys Beer's law in the concentration range of 4-24 µg/ ml, 1-10 µg/ ml and 2-20 µg/ ml for OLM, AML and HCTZ respectively. The proposed method for simultaneous estimation of OLM and AML utilizes the spectrum mode of analysis of Shimadzu double beam UV-visible spectrophotometer (model 1700). The method requires six mixed standard solutions involving scanning between 200 to 400 nm. Sampling wavelengths based upon the direct UV spectroscopic data. There was no interference from tablet excipients was observed in these methods. The values of % RSD and correlation of coefficient for simultaneous determination (Tablet) were found to be (% RSD 0.3302 - 0.9501) and correlation coefficient was 0.9999 for OLM, AMLO and HCTZ. The result of recovery studies for tablet was found to be in the range of 99.47 -100.19%.It indicates that there is no interference due to excipients present in the formulation. It can be easily and conveniently adopted for routine quality control analysis. Statistical analysis proves that, these methods are repeatable and selective for the analysis of OLM, AMLO and HCTZ. It can therefore be concluded that use of these methods can save much time and money and it can be used in small laboratories with accuracy.

Table 1. Regression analysis of calibration curve and summary of validation parameters

Parameters	OLM	AML	HCTZ
Wavelength(nm)	256.5 nm	239 nm	271.5 nm
Beers law limit(µg/mL)	4-24 µg/ mL	1-10µg/ml	2-20µg/ml
Molar absorptivity(L/mol/cm)	2.7196 $x10^4$	2.1758 $x10^4$	2.2212 $x10^4$
Limit of Detection(µg/mL)	0.1055	0.0174	0.0645
Limit of Quantification(µg/mL)	0.3198	0.0527	0.1955
Sandell's sensitivity(µg/cm^2)	0.02047	0.02602	0.01330
Intercept α	0.0015	0.0034	-0.0055
Slope β	0.0488	0.0384	0.0751
Regression equation	Y= 0.0488x + 0.0015	Y= 0.0384x + 0.0034	Y=0.0751x - 0.0055
Correlation coefficient r^2	0.9999	0.9999	0.9999
Standard error	0.0058	0.0018	0.0051

Where, ****y= α + ß x, x is the concentration of the analyte and y is the absorbance value***

Table 2. Results of commercial tablet formulation

Analyte	Label claim(mg/tab)	% label claim Estimated*(Mean ± S. D.)	R.S.D
OLM	20	99.45±0.9449	0.9501
AML	5	98.71±0.3260	0.3302
HCTZ	12.5	100.46±0.6816	0.6784

*Average of six determinations; R.S.D., relative standard deviation

Table 3. Results of recovery study

Component	OLM	RSD	AMLO	RSD	HCTZ	RSD
	Mean ±SD		Mean ±SD		Mean ±SD	
I^{st} level	99.81±0.26	0. 3270	99.03±1.09	0.7512	99.74±1.01	0.7664
II^{nd} level	99.16 ±0.43	0.5423	100.26±1.22	1.1603	99.76±0.17	0.1023
III^{rd} level	99.46±1.31	1.2014	100.39±1.25	1.159	101.08± 0.93	1.0164
	99.47		99.89		100.19	

*Average of three determinations; R.S.D., relative standard deviation

Table 4. Results of repeatability

Analyte	Label claim(mg/tab)	% label claim Estimated*(Mean ± S. D.)	% R.S.D
OLM	20	100.15±0.53	0.642
AML	5	100.47±0.39	0.421
HCTZ	12.5	100.78±0.29	0.314

*Average of nine determinations; R.S.D., relative standard deviation

Table 5. Results of Intraday precision

Time	% label claim Estimated*(Mean ± S. D.)			% R.S.D		
	OLM	AML	HCTZ	OLM	AML	HCTZ
T-1	100.72±0.52	99.91±0.10	100.91±0.28	0.9321	0.9780	0.5720
T-2	99.24±0.25	100.12±0.82	99.77±0.71			
T-3	100.97±0.64	98.34±0.73	100.46±0.43			

*Average of nine determinations; R.S.D., relative standard deviation

Table 6. Results of Interday precision

Day	% label claim Estimated*(Mean ± S. D.)			% R.S.D		
	OLM	AML	HCTZ	OLM	AML	HCTZ
D-1	99.23±0.89	100.47±0.27	100.91±0.36	0.8528	1.2808	0.2688
D-2	100.44±0.71	100.63±0.52	100.37±0.92			
D-3	100.88±0.28	98.34±1.27	100.67±0.14			

Table 7. Results of robustness (Analysis using methanol)

Analyte	Label claim(mg/tab)	% label claim Estimated*(Mean ± S. D.)	% R.S.D
OLM	20	99.46 ±0.38	0.426
AML	5	98.99 ±0.42	0.829
HCTZ	12.5	100.56 ±0.12	0.494

Saminathan J. and Vetrichelvan T. / Vol 1 / Issue 1 / 2011/ 7-14.

Table 8. Results of ruggedness (Different analysts and instruments)

Analyte	Label claim(mg/tab)	% label claim Estimated* (Mean ± S. D.)		% label claim Estimated* (Mean ± S. D.)	
		Analyst 1	Analyst 2	Instrument 1	Instrument 2
OLM	20	99.46 ±0.38	99.76 ±0.24	100.42 ±0.51	100.46 ±0.18
AML	5	98.99 ±0.42	99.58 ±0.68	99.23 ±0.39	98.49 ±0.72
HCTZ	12.5	100.43±0.12	100.56±0.12	99.27±0.27	99.89±0.43

Fig 1.Structure of Olmesartan Medoxomil

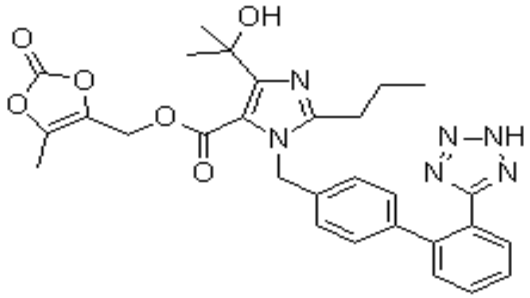


Fig 2.Structure of Amlodipine besylate

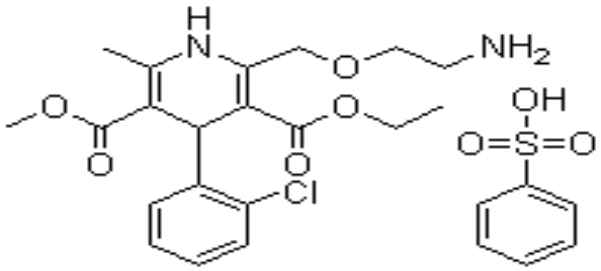


Fig 3. Structure of Hydrochlorothiazide

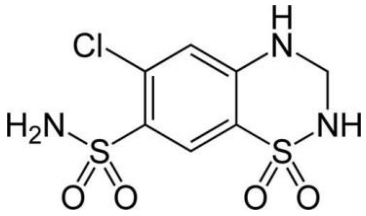


Fig 4. Overlain UV spectra of Olmesartan, Amlodipine and Hydrochlorothiazide

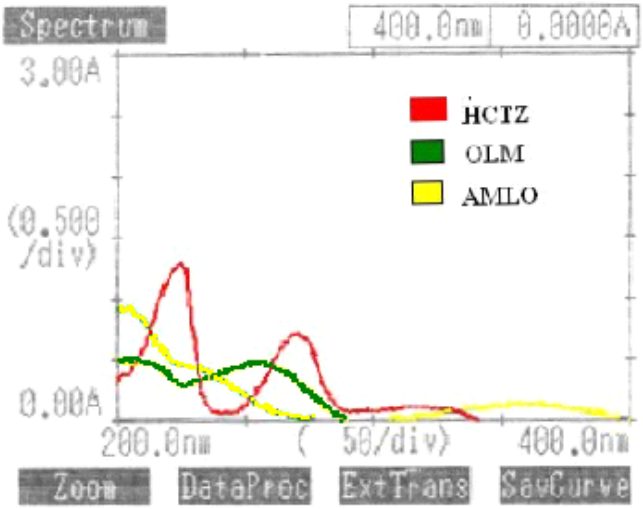


Fig 5.

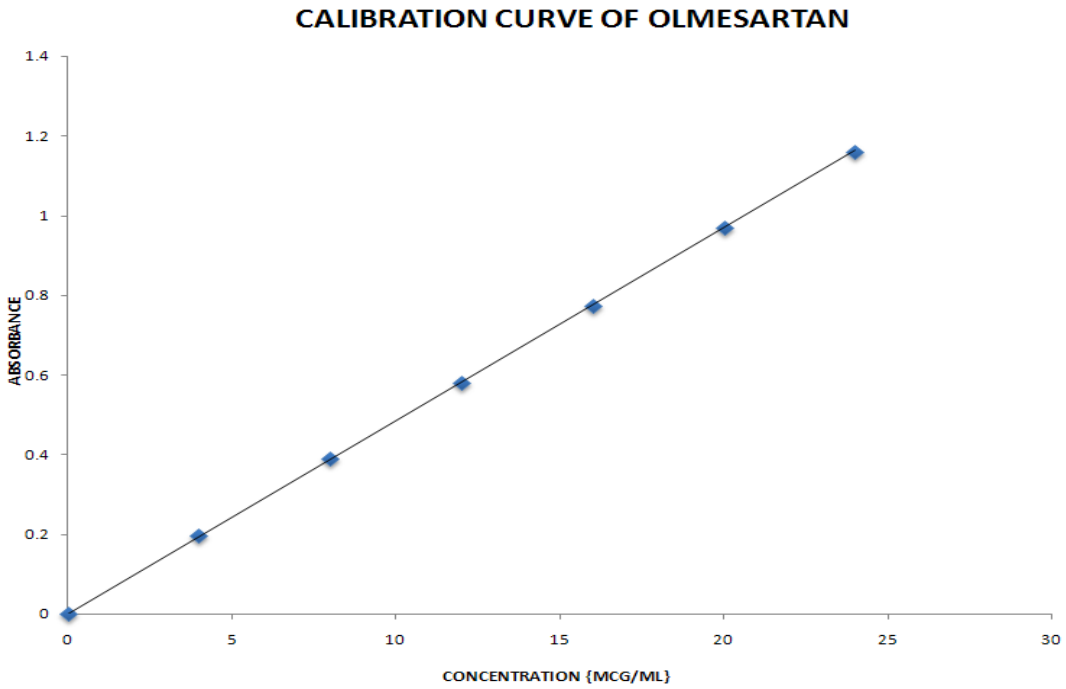

CALIBRATION CURVE OF OLMESARTAN
ABSORBANCE
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
CONCENTRATION {MCG/ML}

Fig 6.

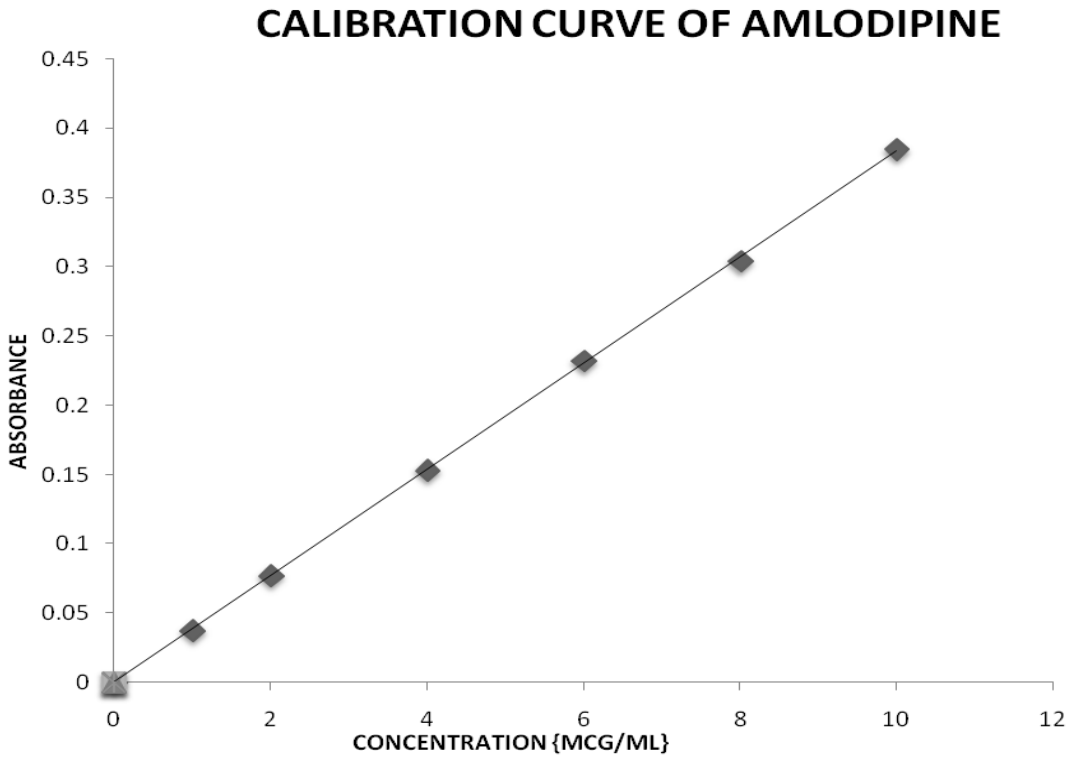
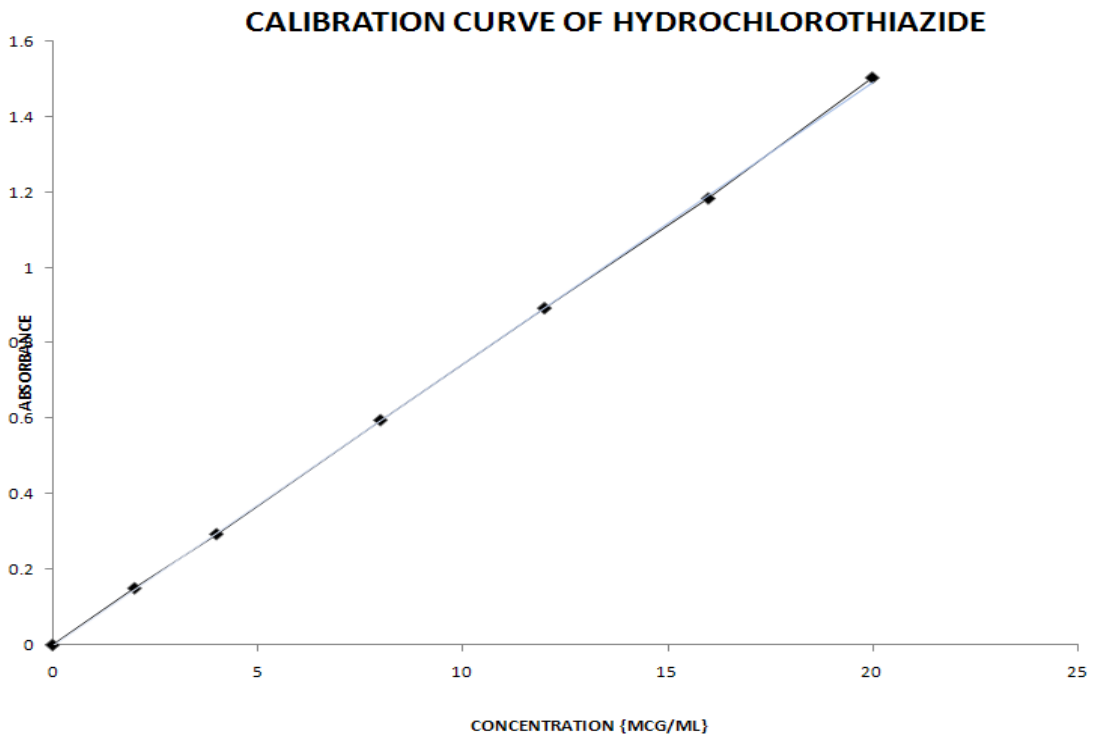

CALIBRATION CURVE OF AMLODIPINE
ABSORBANCE
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
8
10
12
CONCENTRATION {MCG/ML}

Fig 7.



CONCLUSION

The proposed method for simultaneous estimation of Olmesartan medoxomil, Amlodipine besylate and Hydrochlorothiazide in their combined dosage form is quite accurate, precise, yield reproducible, rugged and is validated as per ICH guidelines. Moreover the method is economic, simple and rapid; hence it can be employed for routine analysis in quality control laboratories

ACKNOWLEDGEMENT

The Authors also thankful to the Accent Pharma, Puducherry for providing the pure drugs for the present work. They are also grateful to management of Sakthi Arulthiru Amma and Thirumathi Amma, ACMEC Trust, Melmaruvathur for providing the necessary facilities to carry out this research successfully.

REFERENCES

1. The Merck Index, 23rd ed. Whitehouse Station, New Jersey. 516, 6235.
2. Martindale, Sweetman SC., The complete drug reference. 32nd ed. Pharmaceutical Press. 822, 1999, 907.
3. Black HR. Triple fixed-dose combination therapy: back to the past. *Hypertension*. 54(1), 2009, 19-22.
4. http://www.drugs.com/cons/olmesartan-amlodipine-and-hydrochlorothiazide.html
5. PournimaPatil, Vaishali Barge, Harinath More, SachinPiswikar, spectrophotometric method for simultaneous determination of olmesartanmedoxomil and amlodipine besylate from tablet dosage form, *International Journal of Current Pharmaceutical Research*, 3(2), 2011, 74 -79.
6. A.R.Rote, P.D. Bari Spectrophotometric estimation of olmesartanmedoximil and hydrochlorothiazide in tablet, *Indian Journal of Pharmaceutical sciences*, 72(1), 2010, 111- 113.
7. Neela Manish Bhatia, Snehal Jawaharlal Deshmane, Harinath Nivrutti More and Prafulla Balkrishna Choudhari. Simultaneous Spectrophotometric Estimation of the Amlodipine Besylate and Hydrochlorothiazide. *Asian J Res Chem* 2(4), 2009, 393 - 397.

8. Gaurav Patel, Sanjay Patel, DharmeshPrajapati and Rajendra Mehta, RP-HPLC Method for Simultaneous Estimation of Amlodipine Besylate and Hydrochlorothiazide in Combined Dosage Forms, *Stamford Journal of Pharmaceutical Sciences*,3(1), 2010, 49-53.
9. Shah NJ, Suhagia BN. Development and Validation of a Simultaneous HPTLC Method for the Estimation of Olmesartan medoxomil and Hydrochlorothiazide in Tablet Dosage Form**.** *Indian J. Pharm. Sci***.** 69(6), 2007, 834-836.
10. Dongyang L, Pei H, Nobuko M, Xiaoming L, Li L, Ji J. Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. *J Chromatogr B*. 856(1-2), 2007, 190–197.
11. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. 5th ed., Ghaziabad, 2007, 318, 714.
12. European Pharmacopoeia. 3rd ed. Strasbourg: Council of Europe: 2001, 431.
13. Skoog, Holler, Nieman, Principles of Instrumental Analysis, Ed.5, Harcourt College Publishers, 701-722.
14. Sharma BK. Fundamental principal of Spectroscopy. Instrumentation Method of chemical Analysis. 25th ed. Meerut: Krishna Prakashan Media (P) Ltd. 2006.
15. Beckett AH, Stenlake JB, Ultraviolet- Visible Absorption Spectroscopy. Practical Pharmaceutical Chemistry part-II. 4thed. New Delhi: CBS publishers and Distributers; 2002. 293–296.
16. ICH Q2A; Guidelines on validation of analytical procedure; Definitions and terminology, Federal Register, 60, 1995, 11260.
17. ICH Q2B; Guidelines on validation of analytical procedure; Methodology, Federal Register, 1996, 60, 27464.