Research Article

Development and Validation of Reversed-phase HPLC Technique for Simultaneous Estimation of Amlodipine, Hydrochlorothiazide and Olmesartan in Raw and Tablet Formulation.

Gunasekar Manoharan*, Mohammed Al Bratty, Hassan A. Alhazmi
Jazan University, College of Pharmacy, Pharmaceutical Chemistry Department, P.O. Box 114, Jazan, Saudi Arabia.
***Corresponding author's E-mail:** shekarphd@yahoo.com

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ABSTRACT

A sensitive feasible RP-HPLC method has developed and validated for the analysis of Amlodipine, Hydrochlorothiazide and Olmesartan in raw and tablet formulation. Successful separation of drugs products is developed on a C (18) column reversed-phase using mobile phase composition of Methanol: Phosphate buffer (37:63 v/v). The flow rate was adjusted to 1.1 mL/minute and the absorption maxima were observed at 260 nm utilizing Shimadzu SPD-20A Prominence UV-Vis detector. Good linearity was obtained in the range of 2-10 µg/ ml, 4-20 µg/ml, and 8-40 µg/ ml for Amlodipine, Hydrochlorothiazide and Olmesartan respectively. The HPLC, tablet formulation assay shows percentage purity ranging from 99.90 to 100.07% for Amlodipine, 99.93 to 100.65% for Hydrochlorothiazide and 99.65 to 100.76 for Olmesartan. The mean percentage purity is 100.02%, 100.20% and 100.10 for Amlodipine, Hydrochlorothiazide and Olmesartan respectively. The chromatographic retention time of Amlodipine, Hydrochlorothiazide and Olmesartan was found to be 5.1 and 8.3 and 10.7 minutes respectively. The tailing factor was 0.807, 0.870 and 0.976 for Amlodipine, Hydrochlorothiazide and Olmesartan respectively. The developed method validated according to the ICH guidelines. The method was found to be applicable for determination and validation of Amlodipine, Hydrochlorothiazide and Olmesartan in combined tablet form.

Keywords: Amlodipine (AML), Hydrochlorothiazide (HCZ), Olmesartan (OLM), HPLC and UV.

INTRODUCTION

Multiple therapies are becoming extremely useful in pharmaceutical dosage forms. As the result, numerous and various combinations of drugs are being introduced into the market. Out of these, anti-Hypertensive drugs are one of the mostly prescribed cardiovascular drugs.

Amlodipine is a drug used to lower blood pressure and prevent the pain in the chest. It belongs to a group of drugs known as calcium channel blockers[1,2]. Amlodipine inhibits calcium ion influx across cell membrane. The chemical name of Amlodipine is [2-(Amino ethoxy) methyl] - 4 - (2-chlorophenyl) - 3 - ethoxycarbonyl- 5-methoxycarbonyl-6-methyl 1,4dihydropyridine benzene Sulfonate[2]. Hydrochlorothiazide is a diuretic drug, used to treat high blood pressure and swelling due to fluid buildup. Hydrochlorothiazide often recommended as a first line treatment. Hydrochlorothiazide is combined with other blood pressure drugs as a single tablet or capsule to increase the effectiveness[2,3]. The chemical name of Hydrochlorothiazide is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide[4]. Olmesartan is an angiotensin II receptor antagonist, which is used for the treatment of high blood pressure[5].

Olmesartan blocks the vasoconstrictor effects of angiotensin II by blocking angiotensin II to type I receptor in vascular smooth muscle[6,7]. The chemical name of Olmesartan 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[8,9].

Literature review shows several methods has been developed and reported for AML, HCZ and OLM estimation in biological fluids and there are some methods reported by[10], spectroscopy[11-13], HPTLC HPLC, UPLC and capillary electrophoresis[14,15]. Two methods were reported for estimation of this combination first is UV spectroscopy[16] and the other is HPTLC method[17-19]. Method development of HPLC estimation for this combination is new method will fulfil all requirements of validation according to ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

The working standard of Amlodipine, Hydrochlorothiazide and Olmesartan was purchased from Sigma, UK. The Marketed sample of Tribenzor Strength Amlodipine 10 mg, Hydrochlorothiazide 12.5 mg and Olmesartan 40mg manufactured and marketed by Daiichi Sankyo, purchased from the local Pharmacy, Germany. Methanol HPLC grade was purchased from Merck, Darmstadt, Germany, phosphoric acids purchased from Fisher Scientific (UK).

Instrumentation

HPLC instrumentation and chromatographic condition

HPLC system of Shimadzu LC-20 AT, with an auto sampler (SIL-20AC HT, Shimadzu, Japan) and SPD-10 detector (SPD- M20A, Japan) was used. For data recording the LC-

solution software used. A Zorbax Eclipse Plus, Agilent Technology column (150mm x 4.6mm, 5µm) was used Pore size of the column 95Å.

For degassing mobile phase, power sonic 505 ultrasonic baths (Hwashin technology, Seoul, Korea) was used. By using oven (CTO-20AC) column was maintained at a temperature of 39˚C and 1.1 ml/min was the flow rate.

Analysis was carried over with 20µl injection volume using SPD-10 detection at 260nm. 15 minutes was set as run time.

Preparation of Standard solution for HPLC

Preparation of Mobile phase

Phosphate buffer was prepared using 1.35g of KH_2PO_4 in 1000 ml of HPLC grade water by using phosphoric acid pH adjusted to 6. It was filtered with 0.45µ membrane filters and degassed in an ultrasonic bath for 10 minutes. The ratio of Methanol: phosphate buffer (37:63 v/v).

Preparation of Amlodipine (AML), Hydrochlorothiazide (HCZ), and Olmesartan (OLM), Stock solution

Accurately 1 mg of AML (RS), HCZ (RS) and OLM (RS) was taken separately in 100 ml volumetric flasks and mixed with 25 ml of mobile phase solution and sonicated for 10 minutes and 75ml of mobile phase was added to the mark and cooled to room temperature.

To get the concentration of 2-10 µg/ml of AML, 4-20 µg/ml of HCZ and 8-40 µg/ml of OLM varying quantities of standard stock solution was diluted with mobile phase. All three AML, HCZ and OLM powder freely soluble in methanol and does not have any interference in the absorption peaks.

Preparation of sample solution

10 tablets of marketed sample of Tribenzor weighed accurately and powder equivalent of 10 mg of AML, 12.5 mg HCZ and 40 mg OLM transferred into 25ml volumetric flasks and dissolved with 25 ml mobile phase and the resulting solution was filtered through Whatman 1 filter paper. Further dilutions were made based on the required concentrations.

Method validation

The present method was proceeded to obtain new, sensitive and easy method for simultaneous estimation by HPLC from tablet formulation.

According to the ICH guidelines recommendations the experimental was validated and USP-30 for parameters such as, system suitability, accuracy, precision, linearity and specificity.

System suitability

System suitability parameters like resolution, retention time, tailing factor and column theoretical plates was performed by injecting six replicates of standards and two replicates of sample preparation at a 100% level to cross verify the accuracy and precision of the chromatographic system.

Linearity

The chromatographic method linearity was established by plotting a graph to concentration vs peak area of AML, HCZ and OLM standard and determining the correlation coefficients (R2) of the three compounds. For the linearity studies of 2-10 µg/ml of AML, 4-20 µg/ml of HCZ and 8-40 µg/ml of OLM respectively were injected into the HPLC system. For 60 minutes column was equilibrated with the mobile phase before injection of the solutions.

Accuracy

The recovery experiments show the accuracy of the method. The recovery was performed by adding AML, HCZ and OLM working standards to placebo (excipients mixture) in the range of test concentration (60%, 80% and 100 %) and expressed as percent (%) recovered. Three samples were prepared for each recovery level. The recovery statistical results are within the acceptance range (S.D. < 2.0) value for AML, HCZ and OLM.

Precision

In the proposed method **t**he intraday and interday precision was determined by analyzing the sample responses 4 repeats on the same day and 4 different days of a week for 4 different concentrations of standard solutions of AML, HCZ and OLM. 4-10 µg/ml of AML, 6-20 µg/ml of HCZ and 16-40 µg/ml of OLM respectively and results are represented in terms of % RSD.

Specificity

The analytical method specificity is to measure the compound accurately in presence of interferences like excipients, degradants and matrix components. The HPLC of standard mixture and formulation shows specificity of method. The HPLC method is able to access the analyte in presence of excipients.

Statistical Parameters

The results of assay obtained are subjected to the following statistical analysis, standard deviation, relative standard deviation, coefficient of variation and standard error.

RESULT AND DISCUSSION

The HPLC chromatogram of AML, HCZ and OLM are presented in figure 1, 2 and 3. Wavelength 260nm was selected by scanning all standard drugs over a wide range of wavelength 200-400nm. Linearity was evaluated by plotting peak area as a functional of analyte concentration for AML, HCZ and OLM.

The specific range was determined from linearity studies, for three drugs and found to be 2-10 µg/ml of AML, 4-20 µg/ml of HCZ and 8-40 µg/ml of OLM. The data was analyzed by linear regression least square fit method. The slop, intercept, correlation coefficient and regression

equation were also determined and the data presented in table 1.

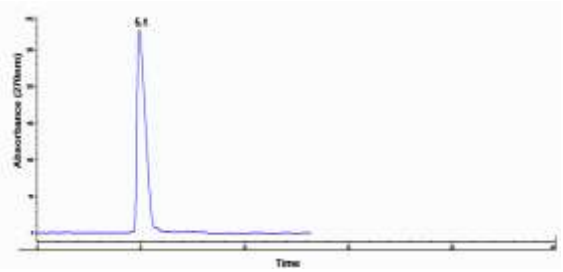

Figure 1: A Typical Chromatogram of Amlodipine Standard

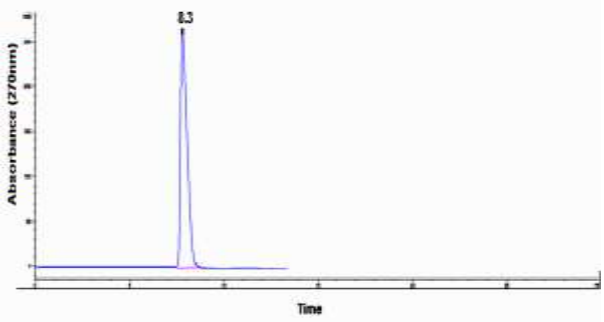

Figure 2: A Typical Chromatogram of Hydrochlorothiazide Standard

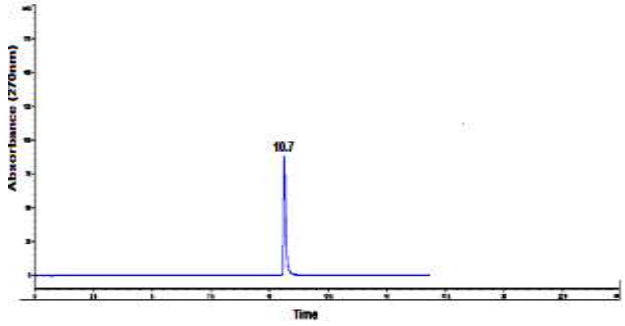

Figure 3: A Typical Chromatogram of Olmesartan Standard

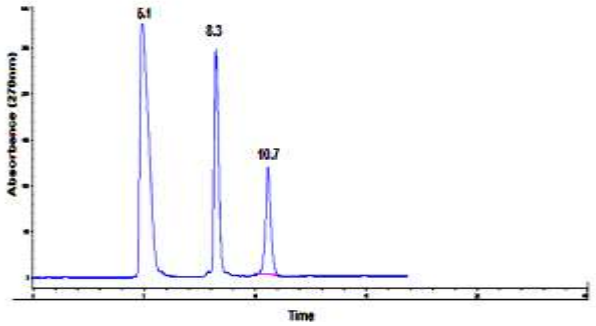

Figure 4: Chromatogram of Amlodipine, Hydrochlorothiazide and Olmesartan in tablet

The system suitability parameters like resolution, tailing factor, retention time and theoretical plates for the developed RP-HPLC method are presented in figure 4; the data are presented in table 2.

The AML, HCZ and OLM chromatographic retention time found to be 5.1 and 8.3 and 10.7 minutes respectively. This is well within the specific limits of 15 minutes. The high – resolution of AML, HCZ and OLM indicates complete separation of the drugs. The tailing factor was found to be 0.807, 0.870 and 0.976 for AML, HCZ and OLM respectively. The peaks are symmetrical and theoretical plates for AML, HCZ and OLM were 7997, 9624 and 7767 respectively which shows the column efficient performance. The quantitative estimation of AML, HCZ and OLM tablet formulation was carried out by RP-HPLC method using Methanol: Phosphate buffer (37:63 v/v) using C18 column as the stationary phase. Chromatogram AML, HCZ and OLM tablet formulation shown in the figure 4. Quantitative estimation (Assay) data of AML, HCZ and OLM presented in table 3. Recovery studies of AML, HCZ and OLM tablet formulation shown in table 4.

Table 1: Results of Statistical parameters

S. No	Parameters	Amlodipine	Hydrochlorothiazide	Olmesartan
1.	Standard deviation (SD)	7.03	4.18	5.42
2.	Relative standard deviation (RSD)	0.00716	0.0112	0.00543
3.	% RSD	0.716	1.121	0.534
4.	Standard error (SE)	0.03286	0.01205	0.03213
5.	Correlation Coefficient (r)	0.9997	0.9994	0.9765
6.	Slope (a)	55.591	28.323	43.41
7.	Intercept (b)	19.106	11.114	17.631
8.	Regression equation Y = (a X + b)	Y = 56.651 X + 18.706	Y = 27.343 X -11.124	Y = 45.791 X + 18.106

Table 2: Results of system suitability parameters

S. No	Parameters	Amlodipine	Hydrochlorothiazide	Olmesartan
1.	Theoretical plates	7997	9624	7997
2.	Tailing factor	0.807	0.870	0.976
3.	Resolution factor	11	11	12
4.	Retention time	5.1	8.3	10.7
5.	Calibration range or Linear dynamic range	2-10	4-20	8-40

Table 3: Quantitative estimation (Assay) data of Amlodipine, Hydrochlorothiazide and Olmesartan

S No	Drug	Label claim (mg/Tab)	Amount found (mg/Tab)	Mean amount found (mg/ Tab)	Percentage purity (% w/w)	Mean percentage purity (% w/w)	% Deviation
1.	AML	10	10.07 10.10 10.01 9.98 10.04	10.04	100.07 100.10 100.01 99.90 100.04	100.02	+ 0.7 +1.0 +0.1 -1.0 +0.4
2.	HCZ	12.5	12.42 12.57 12.73 12.60 12.56	12.57	99.93 100.07 100.23 100.65 100.56	100.20	-0.7 +0.7 +0.2 +0.6 +0.6
3.	OLM	40	40.57 40.70 39.71 39.97 40.44	40.13	100.32 100.47 99.84 99.65 100.76	100.10	+0.3 +0.4 -0.8 -0.6 +0.7

Table 4: Recovery studies of Amlodipine, Hydrochlorothiazide and Olmesartan from tablet formulation

S No	Drug	Amount of Drug present in preanalyzed Sample	Amount of Standard drug (RS) added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery	Mean recovery in Percentage
1.	AML	6	4.00 6.00 8.00	10.03 12.28 14.62	100.23 100.48 99.95	100.22
2.	HCZ	12	6.00 12.00 16.00	17.92 24.08 28.24	99.92 100.43 100.80	100.38
3.	OLM	32	16.00 24.00 32.00	48.52 56.12 64.14	100.21 100.12 100.14	100.15

The tablet formulation shows percentage purity ranging from 99.90 to 100.07% for AML, 99.93 to 100.65% for HCZ and 99.65 to 100.76 for OLM. The mean percentage purity is 100.02% 100.20% and 100.10% for AML, HCZ and OLM respectively. The percentage deviation was found to be -1.0 to +1.0%, -0.7 to +0.7 and -0.8 to +0.7 for AML, HCZ and OLM respectively. The RSD values are below 2% indicating the method precision and the accuracy of the method shown by the low standard error values. This shows a good index of accuracy and reproducibility of the developed method. All the parameters including flow rate, detection wavelength sensitivity was maintained constant.

CONCLUSION

The proposed and developed RP-HPLC method is precise, accurate and sensitive. The method is rapid, reproducible and economical and does not have any interference due to the excipients in the pharmaceutical preparations.

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