ISSN : 0974-7419 Volume 10 Issue 9

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Urgent Communication

ACAIJ, 10(9) 2011 [624-627]

Development and validation of RP-HPLC methods for simultaneous estimation of amlodipine, hydrochlorothiazide and olmisartan medoxomil in tablets formulation

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Received: 16th March, 2011 ; Accepted: 26th March, 2011

ABSTRACT

A reverse phase high performance liquid chromatography methods have been developed for the simultaneous estimation of Amlodipine, Hydrochlorothiazide and Olmisartan Medoxomil from Pharmaceutical product. In reverse phase high performance liquid chromatography analysis was carried out by using 1 % Triethylamine (pH 2.5) :Acetonitrile (65:35 v/v) as the mobile phase and Inertsil ODS 3V (4.6 mm i.d×150 mm x 5 µ) column as stationery phase with detection wavelength of 239 nm. Using 10µL injection volume.

KEYWORDS

Amlodipine besylate;
Hydrochlorothiazide;
Olmisartan medoxomil;
Reverse phase high performance liquid chromatography;
Triethylamine;
O-phosphoric acid.

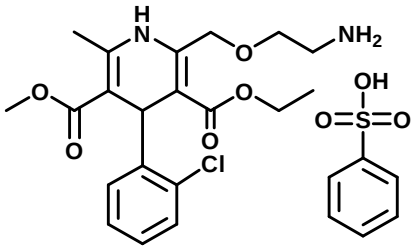
INTRODUCTION

Amlodipine Besylate, Hydrochlorothiazide and Olmesartan Medoxomil is recently introduced in the market as combined tablet dosage form which is widely used in the treatment of hypertension. There is no method reported for simultaneous estimation of Amlodipine, Hydrochlorothiazide and Olmisartan medoxomil from dosage forms by Reverse phase HPLC

Amlodipine besylate (AMLO)

Chemically is Amlodipine besilate; 2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine benzenesulfonate. It is a long acting calcium channel blocker used as an antihypertensive agent.

AMLO structural formula



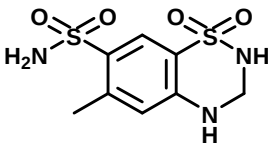
Molecular formula: $C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$
Molecular weight: 567.05

Amlodipine is in a class of drugs called calcium channel blockers. This drug works by relaxing and widening the lumen of the blood vessels, in veins and arteries, making it easier for the heart to pump and reduces its workload.

Hydrochlorothiazide (HCTZ)

6-chloro-1,1-dioxo-3,4-dihydro-2benzo[e][1,2,4]thiadiazine-7-sulfonamide Hydrochlorothiazide is a thiazide diuretic that helps to prevent your body from absorbing too much salt, which can cause fluid retention.

HCTZ structural formula



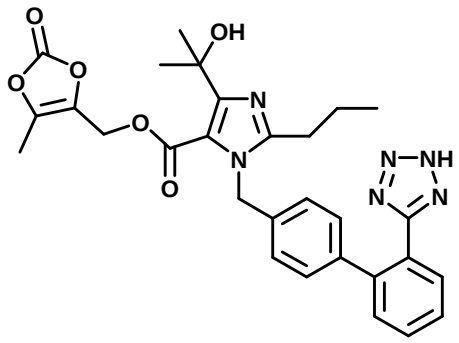
Molecular formula: $C_{29}H_{30}N_6O_6C_7H_8ClN_3O_4S_2$
Molecular weight: 297.7390

Hydrochlorothiazide treats fluid retention (edema) in people with congestive heart failure, cirrhosis of the liver, or kidney disorders, or edema caused by taking steroids or estrogen. This medication is also used to treat high blood pressure (hypertension). Hydrochlorothiazide may also be used for other purposes.

Olmisartan medoxomil (OLM)

Chemically, 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 is an angiotensin II receptor blocker and chemically is used as an antihypertensive agent.

OLM structural formula



Molecular formula: $C_{29}H_{30}N_6O_6$
Molecular weight: 558.59

Olmesartan medoxomil is a drug used to treat high blood pressure (hypertension). It is a type of drug called an angiotensin II receptor blocker. It may be used alone or with other drugs to treat high blood pressure.

MATERIAL AND METHODS

Amlodipine Besilate, Hydrochlorothiazide and Medoxomil reference substances with claimed purity were taken from Precise Pharma (Turbhe, Mumbai) Acetonitrile (HPLC grade), triethyl amine (HPLC grade) and orthophosphoric acid (HPLC grade) were purchased from Merck (Mumbai). All excepients used were of pharmaceutical grade. Mobile phase was filtered using 0.45µm nylon membrane filter made by Millipore (USA).

For high performance liquid chromatographic method System used Agilent Technologies 1200 series with Ezetchrome software for data processing was used. The following optimum conditions were established for quantitative analysis of AMLO, HCTZ and OLM in tablet formulation. A mixture of 1% Triethylamine (pH-2.5 adjusted with ortho phosphoric acid) and acetonitrile in the ratio of (65:35 v/v), column: Inertsil ODS 3V (4.6 mm i.d×150 mm x 5 µ partical size), flow rate: 1.2 ml/min for 10min. detection wavelength: 239 nm, Standard solution of AMLO, HCTZ and OLM 50 µg/ml, 125 µg/ml and 200 µg/ml, respectively were prepared in mobile phase and chromatographed under optimum chromatographic conditions. Sample solutionwere prepared in mobile phase of same concentration that of standard 50:125:200 µg/ml AMLO: HCTZ:OLM respectively. 10 µL of solution was injected and chromatographed under optimum chromatographic condition. A typical chromatogram of AMLO, HCTZ and OLM is shown in Figure 1. The concentration of AMLO, HCTZ and OLM in tablet sample solution was determined by comparing the peak area of the sample with that of standard at 239 nm.

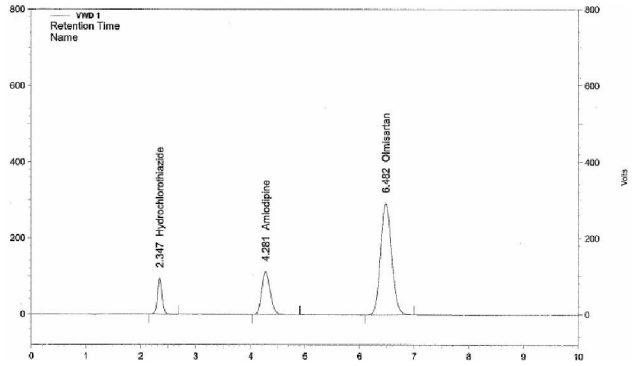


Figure 1 : Standard solution

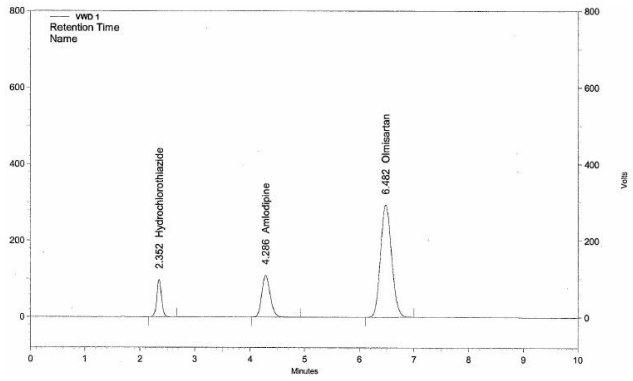


Figure 2 : Sample solution

EXPERIMENTS

Specificity

Solutions of blank, Placebo, standard and sample wee injected separately to prove that the prosoed method is free from any interference at tie retention time of AMLO, HCTZ and OLM.

Method precision

Precision was evaluated by carrying out assay of AMLO, HCTZ and OLM on 6 replicate preparations of tablets powder. Assay and RSD of replicate values of assay was determined.

TABLE 1 : Precision of the proposed HPLC method

Compounds	Level (%)	n	Mean % Assay	% RSD
AMLO	100	6	100.5	1.2
HCTZ	100	6	98.3	0.7
OLM	100	6	98.3	0.5

Intermediate precision

Intermediate precision was evaluated by carrying out assay of AMLO, HCTZ and OLM on 6 replicate preparations of tablets powder. On Different day, on different Instrument. Assay and RSD of replicate values of assay was determined.

Accuracy

The proposed method was evaluated for accuracy by performing recovery of the spiked samples Recovery was carried out by adding AMLO, HCTZ and OLM drug substances in the placebo mixture at various levels to cover a range from about 20% to 300% of the test solution concentration of Working levels.

TABLE 2 : Intermediate precision of the proposed HPLC method

Compounds	Level (%)	n	Mean % Assay	% RSD
AMLO	100	6	99.1	1.7
HCTZ	100	6	100.6	1.6
OLM	100	6	99.6	0.1

TABLE 3 : Accuracy of the proposed HPLC method

Compound	Level (%)	n	Added (µg/mL)	Found (µg/mL)	% Recovery
AMLO	20	3	10	10.1	101.0
	100	3	50	50.1	100.2
	200	3	100	100.2	98.8
	300	3	150	151.5	100.6
HCTZ	20	3	25	25.2	101.1
	100	3	125	126.3	100.4
	200	3	250	250.2	100.5
	300	3	375	400.2	101.0
OLM	20	3	40	39.3	100.5
	100	3	200	199.6	99.5
	200	3	400	386.8	99.6
	300	3	600	587.7	99.5

These samples were analyzed for AMLO, HCTZ and OLM content. The recovered amount at each level was compared with the added amount and % recovery was calculated.

Linearity and range

The linearity of detector response was established in the concentration range from 10.0 µg per mL to 150 µg per mL for AMLO, 25.0 µg per mL to 375.0 µg per mL for HCTZ and 40.0 µg per mL to 600.0 µg per mL for OLM, covering a range from 20% to 300% of the working level concentration.

A graph of peak area response vs. concentration was plotted and Linearity regression coefficient. RSD of six replicates of level one and level seven was determined.

Robustness

Robustness of the proposed method was studied by applying the method for determination of assay of AMLO, HCTZ and OLM Tablets with deliberate small changes in method parameters. For each change assay samples were analyzed in triplicate. And Mean % Assay was calculated.

TABLE 4 : Robustness of proposed HPLC method

Robustness	n	% Assay (Mean)		
		AMLO	HCTZ	OLM
Flow (1.1 ml/Min)	3	100.6	101.7	98.5
Flow (1.3 ml/Min)	3	101.0	102.3	98.6
pH of buffer (pH 2.3)	3	99.8	100.4	99.8
pH of buffer (pH 2.7)	3	100.2	98.8	99.6
Buffer:ACN (67:35)	3	100.0	99.9	100.0
Buffer:ACN (63:35)	3	100.2	100.4	100.1
Buffer:ACN (65:37)	3	100.2	100.5	100.2
Buffer:ACN (65:33)	3	100.1	100.3	100.0

Results

In the proposed method it was found that there is no interference of blank or placebo at the retention time of HCTZ, AMLO, and OLM 2.347, 4.281 and 6.482 Minutes respectively. Shown in figure 1. The developed HPLC method was validated. Validation parameters performed include Specificity, precision (Reproducibility), Intermediate precision, Accuracy, Linearity, robustness. The calibration curve was linear over the concentration range of 10-150 µg/ml for AMLO, 25-375µg/ml for HCTZ and 40- 600 µg/ml for OLM. The correlation coefficient in all three cases was found to be greater than 0.995 which manifests a linear relationship between concentration and Detector responce.

CONCLUSION

The proposed method for simultaneous estimation of AMLO, HCTZ and OLM in their combined dosage form are quit precise, Accurate, reproducible, Linear and rugged. Moreover the method is economic, simple and rapid, hence can be employed for routine analysis in quality control laboratories.

ACKNOWLEDGEMENTS

The authors thanks to Precise Pharma Ltd., Turbhe navi Mumbai; to provide the Necessary supports required for the validation of above HPLC method.

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