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Simultaneous Determination and Validation of Olmesartan Medoxomil, Amlodipine Besilate and Hydrochlorothiazide in Combined Tablet Dosage Form Using RP-HPLC Method

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A simple, accurate and precise RP- HPLC method has been developed for the simultaneous estimation of olmesartan medoxomil (OLME), amlodipine besilate (AMLO) and hydrochlorothiazide (HCTZ) in commercially available tablet formulations. The chromatographic separation was achieved on instrument Shimadzu LC 10 AT VP, Japan, equipped with photodiode array detector (PDA) SPD-10 AVP, attached with a class M 10 A software, (version 1.6) and Phenomenex Luna C_8 (25 cm × 4.6 mm i.d. × 5 µm) column using acetonitrile: phosphate buffer (pH 4 ± 0.1) (40:60 % v/v) as mobile phase at a flow rate of 1.0 mL/min. Quantitation was carried out at 258, 237 and 270 nm for Olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide respectively. The method was validated as per ICH guidelines. The method may be applied for the routine simultaneous laboratory analysis of the above mentioned drugs in tablet dosage form.

Key Words: RP-HPLC, Olmesartan medoxomil, Amlodipine besilate, Hydrochlorothiazide.

INTRODUCTION

Olmesartan, the medoxomil salt of (5-Methyl-2-oxo-1,3-dioxol-4-yl) methyl ester of 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl}-1*H*-imidazole-5-carboxylic acid (Fig. 1), is the ester prodrug of a new generation of effective and orally active angiotensin-II receptor antagonist. These substance blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin-II and one of the most important regulators of blood pressure[1].

Amlodipine besilate (Fig. 1) or 3-ethyl 5-methyl (4RS)-2-[(2- aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4- dihydropyridine-3,5-dicarboxylate benzene sulphonate is a cardiovascular (antianginal), dihydropyridine calcium channel blocker widely used in antihypertensive pharmaceutical formulations[2,3].

Hydrochlorothiazide (Fig. 1) or 6-chloro-3,4-dihydro- 2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which decreases active sodium reabsorption and reduces peripheral vascular resistance[4].

The determination of olmesartan medoxomil from tablet formulation has been carried out by HPLC, capillary electrophoresis and spectrophotometrically, alone or in combination[5,6].

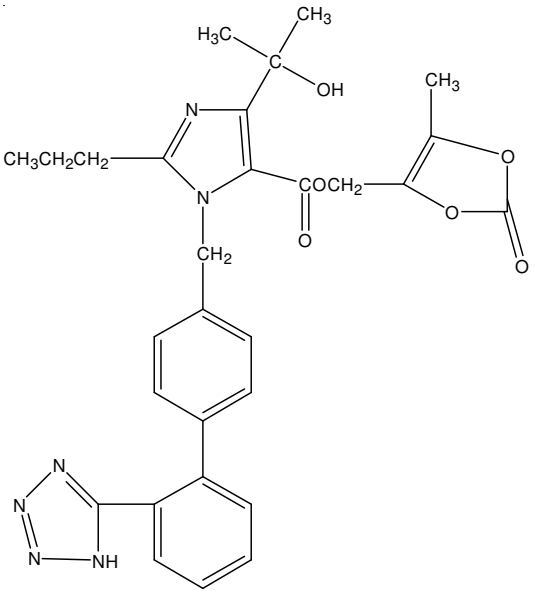
Assay of amlodipine besilate in bulk and in dosage form is official in Indian Pharmacopoeia (2007), British Pharmacopoeia. Several analytical methods have been reported for the determination of amlodipine besilate in dosage form, in biological fluids and in urine using HPLC, LC/MS, CE and UPLC[7-11].

Assay of hydrochlorothiazide in bulk and in dosage form is official in Indian Pharmacopoeia (2007), British Pharmacopoeia. Several analytical procedures have been described for the individual determination of hydrochlorothiazide, most frequently by using spectrophotometric methods and jointly with other drugs using spectrophotometric and HPLC procedures[12-15]. The structure of drugs are shown in Fig. 1.

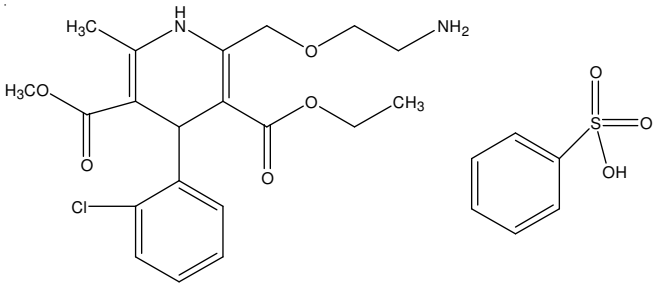
This paper describes a novel method for the simultaneous estimation of olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide in tablets dosage form. The procedure, based on the use of reversed-phase high-performance liquid chromatography, is simple rapid and provides accurate and precise results. The proposed methods were optimized and validated according to current International Conference on Harmonization (ICH) guidelines[16].

EXPERIMENTAL

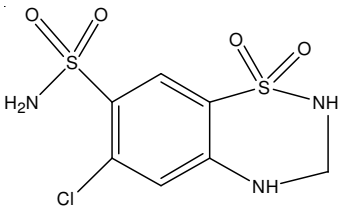
Olmesartan medoxomil was provided by Cipla Ltd. (Mumbai, India), Amlodipine besilate and hydrochlorothiazide were obtained from Ipca Laboratories (Ratlam, India) as gratis



Olmesartan medoxomil (OLME)



Amlodipine besilate (AMLO)



Hydrochlorothiazide (HCTZ)

Fig. 1. Chemical structures of olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide

sample. All the chemicals and reagents used were of HPLC grade and purchased from Merck Ltd. (Mumbai, India). The percentage purity of Olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide were found to be 99.26, 99.90 and 99.41 %, respectively.

Chromatographic system and conditions: The LC system consisted of (Shimadzu LC 10AT VP) gradient pump with universal loop injector (Rheodyne 7725i) of 20 mL injection capacity, photodiode array detector (PDA) SPD-10 AVP and Phenomenex Luna C_8 (25 cm × 5 mm × 4.6 mm i.d.) column at 1.0 mL/min flow rate controlled by a PC work station equipped with software CLASS-Vp (software M-10, version 1.6) (Shimadzu. Tokyo, Japan).

The mobile phase consisted of a mixture of acetonitrile and phosphate buffer (pH 4.0 ± 0.1 adjusted with orthophosphoric acid) (40:60, % v/v). Mobile phase was filtered through a 0.45 µm membrane filter and delivered at a flow rate of 1.0 mL/min.

Standard stock solutions: The equivalent of 10 mg each of Olmesartan medoxomil, amlodipine besilate and 5 mg of hydrochlorothiazide were accurately weighed, different dilutions were prepared for each drug having concentration from 10, 20, 30, 40 and 50 mg/mL for olmesartan medoxomil and amlodipine besilate and 5, 10, 15, 20 and 25 mg/mL for hydrochlorothiazide with mobile phase. Then 20 mL of these solutions were injected into the LC system with the help of Hamilton syringe. The chromatograms were recorded at 258, 237 and 270 nm for olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide, respectively. Peak area was used to prepare calibration curve against their respective concentrations.

Analysis of tablets: As the result of mixed standard analysis found satisfactory, the method was applied for the quantitative study drugs in commercially available tablet. For the preparation of the stock solution of tablet dosage form, 20 tablet of Olmat-AMH were taken and their average weight was determined. 20 tablets were crushed and weighed equivalent to 5 mg of amlodipine besilate was taken in 100 mL volumetric flask and dissolved in 10 mL of mobile phase (acetonitrile and phosphate buffer 40:60, % v/v) (pH 4 ± 0.1) with vigorous shaking for 5 min. The supernatant liquid was transferred to 100 mL of volumetric flask through a whatman # 41 filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100 mL mark. 1 mL of the above solution was diluted up to 100 mL with solvent. Five replicates of sample solutions were prepared and 20 µL of each replicates were injected into the system. The concentrations of these drugs were extrapolated from their respective calibration curves by using the area.

Recovery study: To check the accuracy of the developed method recovery study was carried out as per ICH guideline. Standard solutions of all the three drugs were added equivalent to 80, 100 and 120 % of target drug concentration. Recovery study was carried in triplicate. Precision of the method was checked using three replicates over three concentration levels of within range expressed as % RSD values.

RESULTS AND DISCUSSION

For the RP-HPLC method, chromatographic conditions were optimized to achieve the best resolution and peak shape for olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide. Mobile phase containing acetonitrile:phosphate buffer (pH 4, 40:60 % v/v) was selected as optimal for obtaining well-defined and resolved peaks. The quantitation was carried out at 258, 237 and 270 nm for olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide, respectively at which the best detector response for all the substances were obtained (Fig. 2). Straight line calibration curves were obtained.

The proposed methods were also evaluated in the assay of commercially available tablets containing olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide. Five replicates determination were performed on the accurately weighed amounts of tablets (Table-2). Linearity range was found to be in the range of 8-160, 4-80 and 2-40 mg/mL with limit of quantification of 2.07, 1.57 and 0.96 for olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide,

TABLE-2
RESULTS OF THE COMMERCIAL TABLET ANALYSIS

S. No.	OLME		AMLO		HCTZ	
	Conc. found*		Conc. found*		Conc. found*	
	(µg/mL)	(%)	(µg/mL)	(%)	(µg/mL)	(%)
1	19.82	99.14	4.94	98.94	12.29	98.34
2	20.10	100.50	5.00	100.14	12.32	98.57
3	19.92	99.64	5.09	101.98	12.65	101.25
4	20.08	100.41	5.05	101.07	12.66	101.30
5	20.07	100.37	5.10	102.04	12.43	99.50
Mean	–	100.01	–	100.83	–	99.79
SD	–	0.54	–	1.17	–	1.27
RSD (%)	–	0.53	–	1.16	–	1.27

*20 µg/mL for OLME, 5 µg/mL for AMLO and 12.5 µg/mL for HCTZ, respectively (RSD %, n = 5).

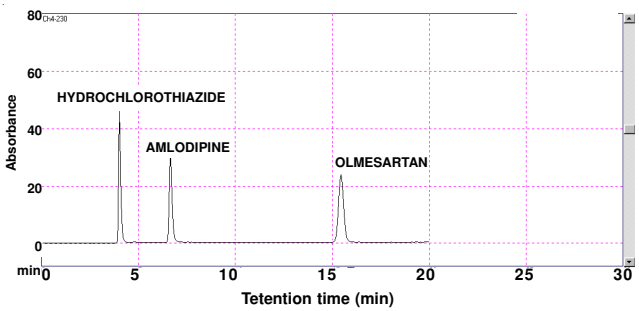


Fig. 2. Chromatogram of olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide in mobile phase

TABLE-1
VALIDATION AND SYSTEM SUITABILITY PARAMETERS FOR RP-HPLC METHOD

Parameters	OLME	AMLO	HCTZ
Linearity range, (µg/mL)	8-160	4-80	2-40
$\lambda_{max.}$ (nm)	258	237	270
Limit of detection, (µg/mL)	0.68	0.51	0.31
Limit of quantitation, (µg/mL)	2.07	1.57	0.96
Theoretical plate number	15404	8692	5905
Retention time (min)	6.69	15.55	4.03
HETP[a]	0.0016	0.0028	0.0042
Tailing factor	1.11	1.54	1.76
Capacity factor (k')	3.59	1.62	_
Resolution	21.89	11.06	_

[a]HETP: Height equivalent to theoretical plate, cm.

respectively, the recovery study showed an acceptable range of variation below RSD of 2 (Table-3). The solution was found to be stable in precision study for long period of time.

TABLE-3
RESULTS OF STATISTICAL VALIDATION OF RECOVERY STUDY

Percentage (%)	Drug	Mean % ± SD	RSD (%)
80	OLME	100.59 ± 1.09	1.09
	AMLO	98.92 ± 0.47	0.48
	HCTZ	100.76 ± 1.06	1.05
100	OLME	100.26 ± 0.34	0.34
	AMLO	101.15 ± 0.98	0.97
	HCTZ	100.92 ± 1.00	0.99
120	OLME	102.72 ± 0.22	0.21
	AMLO	101.25 ± 0.27	0.27
	HCTZ	102.10 ± 0.52	0.50

Conclusion

The validated RP-HPLC method developed here found to be simple, fast, accurate, precise and sensitive. The developed method was validated based on ICH guidelines. Thus, it can be used for routine analysis of olmesartan medoxomil, amlodipine besilate and hydrochlorothiazide in combined tablet dosage form.

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