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RESEARCH ARTICLE

VALIDATED SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESILATE, OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE IN THEIR COMBINED DOSAGE FORMS

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ABSTRACT

A method was developed for the simultaneous estimation of amlodipine besilate, olmesartan medoxomil and hydrochlorothiazide in tablet dosage form by using methanol as solvent. Three wavelength selection method were apply, in which three analytical wavelengths 360 nm, 253 nm, 270 nm were selected for amlodipine besilate ,olmesartan medoxomil and hydrochlorothiazide respectively. Shimadzu 1800 capable of multi component analysis was used for quantification. In this method estimation of amlodipine besylate was carried out by plot a calibration curve absorbance Vs concentration at 360 nm. Where as olmesartan medoxomil and hydrochlorothiazide were estimated by simultaneous equation method. The method was found to be liner at concentration range 5-19 ppm 4-32 ppm and 2-23 ppm for amlodipine besylate ,olmesartan medocxomil and hydrochlorothiazide with r^2 0.998, 0.999 and o.996 respectively. The method was found to be accurate with % recovery 99.86 % for amlodipine besylate, 100.30 % for olmesartan medoxomil and 99 % for hydrochlorothiazide. precision was found 1.48, 1.16 and 1.07 intra day and 0.99, 2.04 and 1.23 inter day for amlodipine besylate ,olmesartan medoxomil and hydrochlorothiazide respectively. LOD was found 0.21, 0.22 and 0.07 and LOQ was found 0.66, 0.60 and 0.30 respectively.

Key words: *amlodipine besylate, olmesartan medoxomil and hydrochlorothiazide.*

INTRODUCTION

Amlodipine besylate (AMLO) is chemically [3-ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-methyl-1-dihydropyridine-3, 5-dicarboxylate benzenesulfonate[1.] AMLO is long-acting calcium channel blocker used as an anti-hypertensive and in the treatment angina and arrhythmias[2-3.] AMLO is official drug in IP. Olmesartan medoxomil as chemically it 2, 3-dihydroxy-2-butenyl4-(1-hydroxy-1-ethylethyl)-2-propyl-1-[*p*-(*o*-1*H*-tetrazol-5-lphenyl) benzyl] imidazole-5-carboxylate, cyclic-2, 3-carbonate. OLME is an angiotensin II receptor blockers used as an antihypertensive agent[4]. Hydrochlorothiazide (HCTZ) is chemically 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide-1,1-dioxide. HCTZ used as diuretic agent[5]. Literature survey reveal that there were no HPLC, UV method was developed for this combination of drugs hence we developed sensitive simultaneous spectrophotometric method for the same combination[6-14].

MATERIALS AND METHODS

Pure drug of amolodipine besylate, olmesartan medoxomil and hydrochlorothizide obtained as gift sample from torrent pharmaceuticals. The commercially available tablet OLMET-AMH (label claim: AMLO 5 mg, OLME 20 mg and HCTZ 12.5 mg) was procured from local market. After assessing the solubility of drugs in different solvent methanol was used as common solvent for developing spectral characteristics. Extraction were carried out by ethyl acetate. All the reagents and chemicals used were of analytical grade.

Instruments

Shimadzu UV 1800 were used with software of UV probe.

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Solvent selection

AMLO and HCTZ were soluble in water but OLME were insoluble in water, where as all three drugs were soluble in methanol so methanol used as solvent.

Preparation of Standard Stock Solution

The standard stock solution of AMLO, OLME and HCTZ was prepared by weighed accurately 10 mg of each drugs and transfer to 100 ml volumetric flask and 25 ml methanol was added to it and sonicate for 5 minute and volume was made up to 100 ml with methanol to get the standard stock solution of 1000 ppm. Aliquots of this standard stock solution were diluted up to 10 ml with methanol in order to obtain a concentration range of 5 to 19 ppm for AMLO 4 to 32 ppm for OLME and 2 to 23 ppm for HCTZ.

Preparation of sample solution

Ten tablets were weighed and crushed to fine powder. Weight powder equivalent to average weight and transfer to 100 ml beaker. Ethyl acetate (30 ml) was added to it and sonicated for 20 min. Ethyl acetate evaporated by heating on hot plate. Residue was washed with 50 ml methanol and transferred to 100 ml volumetric flask. The solution was again sonicated for 10 minutes and filtered through whatman filter paper No. 41. Residue was washed with 5ml of methanol twice. Filtrate and washings were combined in another 100 ml volumetric flask and volume was adjusted up to the mark with methanol to obtain 50 µg/ml AMLO, 200 µg/ml OLME and 125 µg/ml HCTZ. Appropriate aliquot (1 ml) was taken in a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of AMLO (5 µg/ml), OLME (20 µg/ml) and HCTZ (12.5 µg/ml) respectively.

Estimation of AMLO, OLME and HCTZ in Tablet Dosage Forms

Overlain spectra suggest that AMLO, OLME and HCTZ showed absorbance maximum at 360 nm, 253 nm, 270 nm respectively. It was observe that OLME and HCTZ does not show absorbance at 360 nm where as AMLO show absorbance. So AMLO was estimated 360 nm by plotting a calibration curve.

OLME (λmax -253 nm) and HCTZ (λmax-270 nm) were estimated by simultaneous equation method, using the equation are as follows:

$$C_X = \left(\frac{A_1 a_{y^2} - A_2 a_{y^1}}{a_{x^1} a_{y^2} - a_{x^2} a_{y^1}} \right) \quad (1)$$

$$C_X = \left(\frac{A_2 a_{x^1} - A_1 a_{x^2}}{a_{x^1} a_{y^2} - a_{x^2} a_{y^1}} \right) \quad (2)$$

Where Cx and Cy are the concentration of OLME and HCTZ.

Ax1 and ax2 are the absorptivity of OLME at 253nm and 270 nm.

Ay1 and ay2 are the absorptivity of HCTZ at 253 nm and 270 nm.

A1 and A2 absorbance of sample at 253 nm and 270 nm.

RESULTS AND DISCUSSION

From the literature it was found that AMLO, OLME and HCTZ are highly soluble in methanol. Moreover there was no shift in the absorbance maxima of all three drugs using methanol as a solvent. Hence methanol was selected as solvent for the study.

From overlain spectra of AMLO, OLME and HCTZ at different concentrations, it is clear that at 360 nm different concentrations of AMLO possess significant absorbance whereas OLME and HCTZ possess zero absorbance. And at 253 nm OLME and 270 nm OLME and HCTZ showed absorbance. Considering above fact, wavelength 360 nm, 253 nm and 270 nm were selected for the estimation of AMLO, OLME and HCTZ respectively. (fig. 1).

Calibration curves for AMLO, OLME and HCTZ were plotted between absorbance vs concentration at 360, 253 and 270 nm respectively. The following equations for straight line were obtained for AMLO, OLME and HCTZ.

Linear equation for AMLO at 360 nm, y = 0.009 xs + 0.028……*Eq. 1.*

Linear equation for OLME at .253 nm, y = 0.0372 xs + 0.0214……..…*Eq. 2.*

Linear equation for HCTZ at 270 nm, y = 0.0534 xs + 0.0179……*Eq. 3.*

The method was found to be linear over the entire calibration range studied with the r^2 values of 0.998, 0.999 and 0.996 for AMLO, OLME and HCTZ. (table1) The linear range, correlation coefficient, detection limit and standard deviation for AMLO, OLME and HCTZ by spectrophotometric method. Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 99.81–00.05 % for AMLO, 99.12–99.60 % for OLME and 99.57–98.78% for HCTZ (table 2) Precision was calculated as repeatability for AMLO, OLME and HCTZ. The method was found to be precise with % CV of 1.42 –1.63 for intraday (n=3) and 1.38–0.65 for interday (n=3) for AMLO, and % CV of 1.96–0.97 for intraday (n=3) and 0.77–2.02 for interday (n=3) for OLME, and % CV of 1.77 – 0.82 for intraday (n=3) and 1.27–1.34 for interday (n=3) for HCTZ. (table 3-5)

The LOD for AMLO and OLME and HCTZ were found to be 0.21µg/ml and 0.22 µg/ml and 0.07 µg/ml respectively. The LOQ for AMLO, OLME and HCTZ was found to be 0.66 µg/ml, 0.60 µg/ml and 0.30 µg/ml respectively.

Marketed formulation (olmet-AMH) was analyzed by the proposed method and assay result was comparable with corresponding labeled amount.(table 10) Percentage estimation for AMLO, OLME and HCTZ in tablet dosage form was 101.25 %, 99.02 % and 99.28 % by this method with standard deviation < 2.

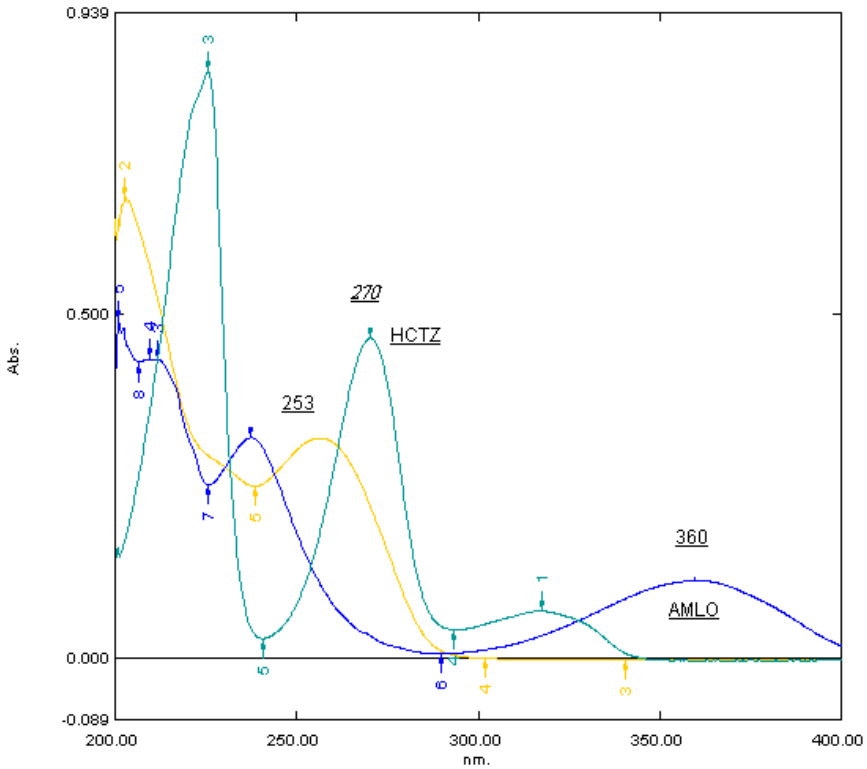

Figure – 1: overlain spectra of AMLO, OLME and HCTZ.

Table - 1: optical characteristics data and validation parameter

Parameter	values		
	AMLO	OLME	HCTZ
Working λ max	360 nm	253 nm	270 nm
Beer's law limit(mcg/mL)	5-19	4-32	2-23
Absorptivity	125.9479	140.1716	555.7481
Correlation coefficient	0.998	0.999	0.996
Intercept	0.028	0.021	0.017
Slope	0.009	0.037	0.053
LOD µg/ml	0.21	0.22	0.07

LOQ µg/ml	**0.66**	**0.60**	**0.30**
Intera-day (precision)	**1.48**	**1.16**	**1.07**
Inter-day (precision)	**0.99**	**2.04**	**1.23**
Accuracy : (recovery)			
10%	**99.81**	**99.12**	**99.57**
30%	**96.74**	**101.69**	**98.65**
50%	**100.50**	**99.60**	**98.78**

Table - 2: Determination of Accuracy of AMLO, OLME and HCTZ

Amt of sample			**Amt of std drug added (% of sample)**			**% Recovery**		
AMLO (µg/ml)	**OLME (µg/ml)**	**HCTZ (µg/ml)**	**AMLO %**	**OLME %**	**HCTZ %**	**AMLO %**	**OLME %**	**HCTZ %**
5	20	12.5	10 %	10 %	30 %	99.81 %	99.12 %	99.57 %
5	20	12.5	10 %	10 %	30 %	96.74 %	101.69 %	98.65 %
5	20	12.5	10 %	10 %	30 %	100.05 %	99.60 %	98.78 %

Table – 3: Precision data for AMLO at 360 nm

Conc. (µg/ml)	**Intraday (n =3)**	**%CV**	**Inter-day (n =3)**	**%CV**
5	0.087333 ± 0.001247	1.42	0.089077 ± 0.001234	1.38
10	0.175 ± 0.002449	1.39	0.176667 ± 0.0017	0.96
15	0.274667 ± 0.004497	1.63	0.276556 ± 0.001812	0.65

Table – 4: Precision data for OLME at 253 nm

Conc. (µg/ml)	**Intraday (n =3)**	**%CV**	**Inter-day (n =3)**	**%CV**
5	0.17333 ± 0.003399	1.96	0.174739 ± 0.007736	0.77
11	0.37331 ± 0.002055	0.55	0.380178 ± 0.012923	1.29
20	0.6693 ± 0.006549	0.97	0.679109 ± 0.020203	2.02

Table – 5: Precision data for HCTZ at 270 nm

Conc. (µg/ml)	**Intraday (n =3)**	**%CV**	**Inter-day (n =3)**	**%CV**
4	0.277667 ± 0.004922	1.77	0.275715 ± 0.003515	1.27
8	0.545333 ± 0.003399	0.62	0.553588 ± 0.06128	1.10
12	0.743667 ± 0.006128	0.82	0.756243 ± 0.010148	1.34

Table – 6: Repeatability data for AMLO at 360 nm

Conc.	7 µg/ml	9 µg/ml	11 µg/ml	13 µg/ml	15 µg/ml	17 µg/ml
	0.094	0.112	0.136	0.156	0.174	0.191
	0.092	0.111	0.135	0.154	0.175	0.194
	0.092	0.112	0.137	0.153	0.174	0.192
	0.091	0.115	0.134	0.152	0.172	0.191
	0.091	0.113	0.136	0.151	0.173	0.192
mean	0.0926	0.1126	0.1356	0.1532	0.1736	0.192
S.D.	0.001817	0.001517	0.00114	0.001924	0.00124	0.001225
%RSD	1.96176	1.34687	0.840837	1.255573	0.656783	0.637888

Table -7: Repeatability data for OLME at 253 nm

Conc.	8 µg/ml	12 µg/ml	16 µg/ml	20 µg/ml	24 µg/ml	28 µg/ml
	0.310	0.475	0.622	0.748	0.931	1.053
	0.311	0.478	0.616	0.744	0.932	1.055
	0.312	0.476	0.631	0.735	0.925	1.056
	0.314	0.470	0.626	0.744	0.922	1.046
	0.315	0.471	0.627	0.757	0.939	1.044
mean	0.3124	0.474	0.6244	0.7456	0.9298	1.0508
S.D.	0.002074	0.003391	0.005684	0.007956	0.006611	0.00545
%RSD	0.663779	0.715436	0.910203	1.0670877	0.71097	0.518631

Table - 8: Repeatability data for HCTZ at 270 nm

Conc.	5 µg/ml	8 µg/ml	11 µg/ml	14 µg/ml	17 µg/ml	20 µg/ml
	0.272	0.467	0.626	0.766	0.949	1.044
	0.276	0.477	0.639	0.777	0.950	1.057
	0.277	0.461	0.640	0.775	0.955	1.048
	0.281	0.460	0.623	0.762	0.949	1.053
	0.282	0.471	0.622	0.759	0.943	1.055
mean	0.2776	0.4672	0.630	0.7678	0.9492	1.0516
S.D.	0.004037	0.007085	0.008803	0.007918	0.004266	0.005177
%RSD	1.454368	1.516523	1.397366	1.031302	0.449446	0.492285

Table - 9: Solvent Suitability Study

Time	**ABSORBANCE**			**RESULT%**		
	AMLO	**OLME**	**HCTZ**	**AMLO**	**OLME**	**HCTZ**
0 hr.	0.076	0.944	1.202	106.66%	100.75%	98.72%
4.0 hrs.	0.074	0.943	1.202	102.22%	99.02%	98.72%
8.0 hrs.	0.075	0.944	1.201	104.44%	100.75%	98.56%
24 hrs.	0.076	0.943	1.201	106.66%	99.02%	98.56%

Table - 10: Assay Results of Marketed Formulation.

formulation	**Actual concentration µg/ml**			**Amount obtained µg/ml**			**%**		
	Amlo	Olme	Hctz	Amlo	Olme	Hctz	Amlo	Olme	Hctz
tablet	5	20	12.5	5.06	19.80	12.41	101.25	99.02	99.28

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