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Development of Spectrophotometric Method for Quantitative Estimation of Amlodipine Besylate, Olmesartan Medoxomil and Hydrochlorthiazide in Tablet Dosage Form

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Abstract

A new, simple, accurate, precise and reproducible UV spectrophotometric method is being developed for the simultaneous estimation of amlodipine besylate, olmesartan medoxomil and hydrochlorthiazide in tablet dosage form. The stock solutions were prepared in methanol. The λ_{max} for amlodipine besylate, olmesartan medoxomil and hydrochlorthiazide were 238.5nm, 256.5nm and 271.5nm respectively. The amlodipine besylate, olmesartan medoxomil and hydrochlorthiazide obeyed Beer's law in concentration range of 5-25µg/ml, 6-30µg/ml and 5-25µg/ml respectively. Results of analysis of simultaneous equation method were analyzed and validated for various parameters according to ICH guidelines.

Keywords: Simultaneous equation method; Amlodipine besylate; Olmesartan medoxomil; Hydrochlorthiazide

Introduction

Amlodipine besylate (Figure 1a) [1] is 3-Ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate is a calcium channel blocker and widely used in the treatment of hyprtension [5]. Olmesartan medoxomil (Figure 1b) [1] is [2,3-dihydroxy-2-buten yl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl]imidazole-5-carboxylate, cyclic 2,3-carbonate] an angiotensin II receptor blocker (ARB). Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle [5] and Hydrochlorthiazide (Figure 1c) [1] is 6-chloro-3, 4-dihydro-2*H*-1,2,4-benzothiadiazine-7- Sulphonamide 1, 1-dioxide is diuretic and antihypertensive drug, which inhibits the reabsorption of sodium and calcium at the beginning of distal convoluted tubules [5]. Amlodipine besylate is official in IP [2] and BP [3] and Hydrochlorthiazide is official in IP [2], BP [3] and USP [4]. Literature survey revealed that various methods such as UV [9,10,15,16,19,20], HPLC [11,17], HPTLC [12,18], LC-MS/MS [13] and UPLC [14] are available in single and combination with other drugs. However, no spectrophotometric method has yet been reported for simultaneous estimation of amlodipine besylate, olmesartan medoxomil and hydrochlorthiazide in tablet dosage forms. Hence, an attempt has been made to develop and validate in accordance with ICH guidelines [7,8].

Materials and Methods

Chemicals

Pharmaceutically pure sample of amlodipine besylate was obtained from Sun pharmaceuticals, Silvasa(GJ), olmesartan medoxomil was obtained from Plathico pharma Ltd. Dewas and hydrochlorthiazide was obtained from Matrix laboratory Mumbai as gift samples along with there analytical reports. Methanol AR grade was obtained from Merck chemical division, Mumbai and Commercial tablet of amlodipine besylate (5mg), olmesartan medoxomil (20mg) and Hydrochlorthiazide (12.5mg), Olmat-AMH (Micro labs) were procured from the local drug market.

Instrument

A double beam UV-visible spectrophotometer (SHIMADZU, Japan), model UV-1700 PC was used. The software employed was UV probe version 2.33. The spectra was recorded over range 200-400nm against solvent in 1 cm quarts cells.

Standard solution preparations

Accurately weighed 100mg of amlodipine, olmesartan and hydrochlorthiazide were transferred into 100 ml volumetric flasks separately and dissolved in 50 ml of methanol and then volume was made up to 100 ml with methanol to get a concentration of 1000 µg/ml for all three drugs. Standard stock solution (1000 µg/ml) was further diluted with methanol to obtain 5-25µg/ml for amlodipine, 6-30µg/ml for olmesartan and 5-25µg/ml for hydrochlorthiazide.

Study of spectra and selection of wavelength

All three drugs were scanned over the range of 200-400 nm and overlay spectra was observed. While studding the overlay spectra it was observed that amlodipine shows maximum absorbance at 238.5nm, olmesartan shows maximum absorbance at 256.5nm and hydrochlorthiazide shows peaks at 271.5nm and 322nm. It was observed that hydrochlorthiazide is interfering with amlodipine and olmesartan at absorbance maxima but difference in absorbance maxima is sufficient and spectral characteristics are such that all three drugs can be simultaneously estimated by simultaneous equation method (Figure 2).

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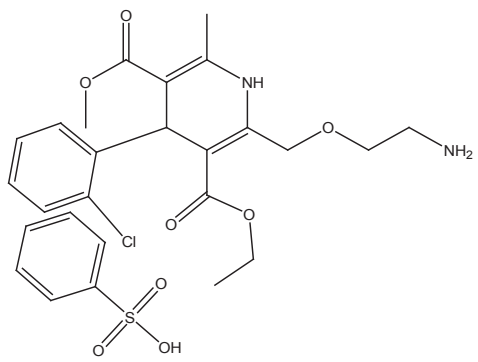

Figure-1a

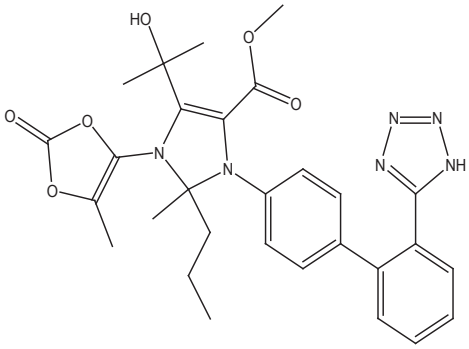

Figure-1b

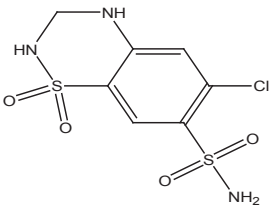

Figure-1c

Figure 1: (a) Structure of amlodipine besylate, (b) Structure of olmesartan medoxomil and (c) Structure of hydrochlorthiazide.

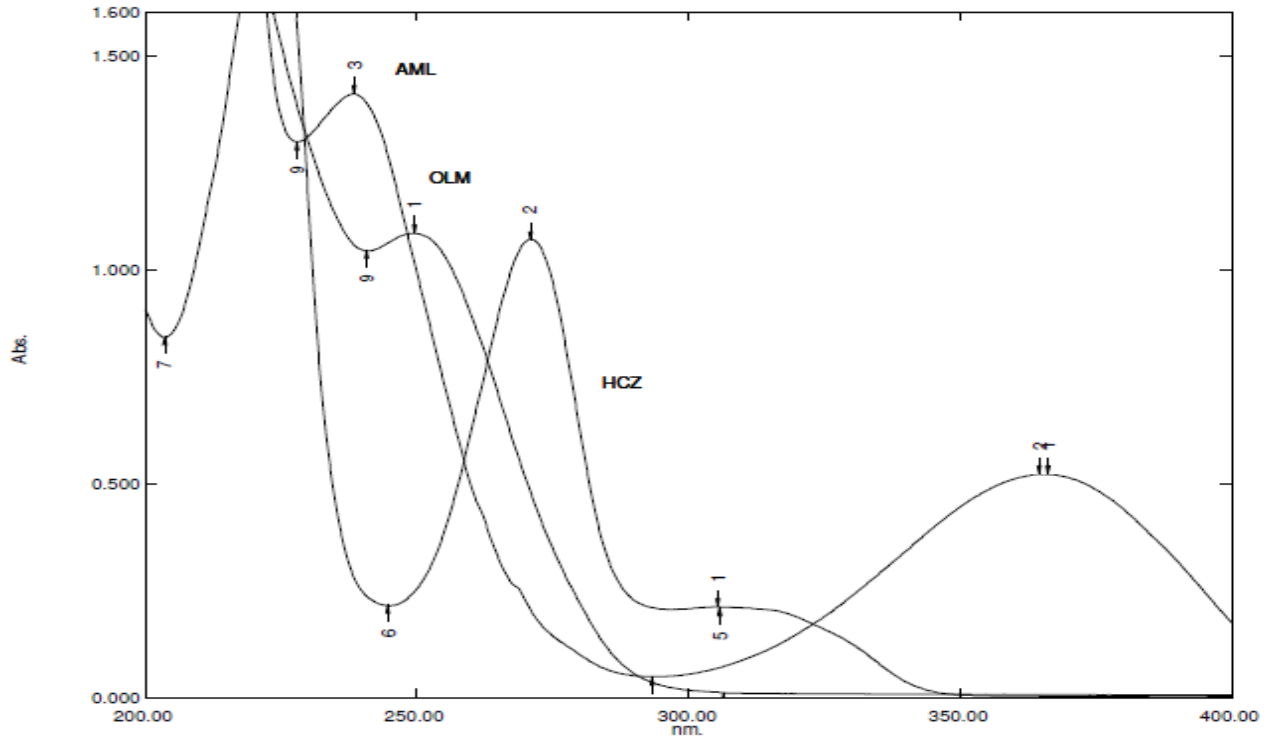

Figure 2: Overlay spectra of amlodipine, olmesartan and hydrochlorthiazide.

The concentration of all three drugs in mixture can be calculated by using following eqns, $C_{AML} = A_1 (a_{y2}a_{z3}-a_{y3}a_{z2})-A_2 (a_{y1}a_{z3}-a_{y3}a_{z1}) +A_3 (a_{y1}a_{z2}-a_{y2}a_{z1})/a_{x1} (a_{y2}a_{z3})-a_{x2} (a_{y1}a_{z3}-a_{y3}a_{z1}) +a_{x3} (a_{y1}a_{z2}-a_{y2}a_{z1})$….. (1), $C_{OLM} = A_1 (a_{x2}a_{z3}-a_{x3}a_{z2})-A_2 (a_{x1}a_{z3}-a_{x3}a_{z1}) +A_3 (a_{x1}a_{z2}-a_{x2}a_{z1})/a_{y1} (a_{x2}a_{z3})-a_{y2} (a_{x1}a_{z3}-a_{x3}a_{z1}) +a_{y3} (a_{x1}a_{z2}-a_{x2}a_{z1})$….. (2), $C_{HCZ} = A_1 (a_{x2}a_{y3}-a_{x3}a_{y2})-A_2 (a_{x1}a_{y3}-a_{x3}a_{y1}) +A_3 (a_{x1}a_{y2}-a_{x2}a_{y1})/ a_{z1} (a_{x2}a_{y3})-a_{z2} (a_{x1}a_{y3}-a_{x3}a_{y1}) +a_{z3} (a_{x1}a_{y2}-a_{x2}a_{y1})$….. (3), Where A_1, A_2 and A_3 are the absorbance values of mixture/tablet solution. $a_{x1,}$ $a_{x2,}$ a_{x3} are the absorptivities of amlodipine at 238.5nm, 256.5nm and 271.5nm respectively. A_{y1}, a_{y2} and a_{y3} are absorptivities of olmesartan 238.5nm, 256.5nm and 271.5nm respectively. A_{z1}, a_{z2} and a_{z3} absorptivities of hydrochlorthiazide 238.5nm, 256.5nm and 271.5nm respectively. C_{AML}, C_{OLM} and C_{HCZ} are concentration of amlodipine, olmesartan and hydrochlorthiazide respectively.

The absorbtivity of all three drugs were calculated by equation (A= abc).

$\mathbf{ax_1}$	0.0366	$\mathbf{ay_1}$	0.0386	$\mathbf{az_1}$	0.0070
$\mathbf{ax_2}$	0.0127	$\mathbf{ay_2}$	0.0483	$\mathbf{az_2}$	0.0296
$\mathbf{ax_3}$	0.0045	$\mathbf{ay_3}$	0.033	$\mathbf{az_3}$	0.0715

The equation are:

$C_X = A_1 (0.0024763382) - A_2 (0.002528319) + A_3 (0.00080425) /$

0.00009798424

$C_Y = A_1 (0.00077485) - A_2 (0.0025854) + A_3 (0.00099446) / 0.00005700193$

$C_Z = A_1 (0.000202669) - A_2 (0.0010371378) + A_3 (0.001278658) / 0.00006708112$

Preparation for analysis of tablet formulation

Twenty tablets were taken and their average weight was determined. They are crushed to fine powder; amount equivalent to 5 mg of amlodipine was taken in 100-ml volumetric flask. The olmesartan and hydrochlorthiazide present in this amount of tablet powder was 20mg and 12.5mg, the ratio of all three drugs was 5:20:12.5. This was than dissolve in 50 ml of methanol by sonication for about 10 minutes. The volume is made upto the mark by methanol and filtered by Whatmann filter paper (no.41) and the filtrate was used to prepare samples of different concentration. Now all the tablet samples was scanned in multi photometric mode and the concentration of all three drugs were obtained from the equation. Results of tablet analysis are reported in Table 1.

Validation of Method

As per ICH guideline the method is validated and following parameters were evaluated.

Linearity

Linearity of the method was determined by diluting the stock solution to give a concentration range of 5-25µg/ml for amlodipine, 6-30µg/ml for olmesartan and 5-25 µg/ml for hydrochlorthiazide. The calibration curve was constructed between concentration verses absorbance.

Precision

Precision was determined by repeatability, Intermediate precision and reproducibility of all three drugs. Repeatability indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The reproducibility is expressed in laboratory to laboratory variation.

Accuracy (% recovery)

To a preanalyzed tablet solution a definite concentration of pure drug was added (80%, 100% and 120% level) and then recovery was studied. A preanalyzed tablet solution containing 5µg/ml of amlodipine 20µg/ml of olmesartan and 12.5µg/ml of hydrochlorthiazide were taken in 10ml volumetric flasks and known concentrations of pure drug solution was added to them, which were prepared from standard stock solution of amlodipine, olmesartan and hydrochlorthiazide. It was repeated at 5 concentration and 3 replicate level.

S. No	DRUG	MEAN	S.D.	%COV	Std. Error
1.	AML	96.05	0.094	0.095	0.092
2.	OLM	98.85	0.145	0.151	0.095
3.	HCZ	98.7	0.23	0.246	0.224

*SD is standard deviation, AML is amlodipine besylate, OLM is olmesartan medoxomil and HCZ is hydrochlorthiazide

Table 1: Result of Tablet formulation.

S.NO.	PARAMETER	AML	OLM	HCZ
1.	Working λ	238.5nm	256.5nm	271.5nm
2.	Beer's law limit (µg/ml)	5-25	6-30	5-25
3.	Correlation Coefficient (r^2)*	0.9997	0.9998	0.9998
4.	Slope (m)*	0.046	0.043	0.072
5.	Intercept (c)*	0.002	0.003	-0.002

* Value of five replicate and five concentrations

Table 2: Result of Linearity of AML, OLM AND HCZ.

Robustness

As per ICH norms, small, but deliberate variations by altering the pH and / or concentration of the solvent were made to check the methods capacity to remain unchanged. The change was made in the ratio of solvent. Instead of 100%, 95% methanol was used as solvent.

Results and Discussion

The simultaneous equation method for estimation of amlodipine, olmesartan and hydrochlorthiazide in tablet dosage form was found to be simple, pricise, accurate and reproducible. The solvent used was 100% methanol and do not shows any significant interference in the spectrophotometric assay of all three drugs.

Linearity

The proposed method was found to be linear in the range of 5-25, 6-30 and 5-25 µg/ml with correlation coefficient 0.9997, 0.9998, and 0.9998 for amlodipine, olmesartan and hydrochlorthiazide respectively. Result of linearity study shown in Table 2.

Precision

Repeatability: The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25 µg/ml for amlodipine and hydrochlorthiazide and 6, 12, 18, 24 and 30µg/ml for olmesartan indicates the precision under the same operating condition over short interval time.

Intermediate precision: Intermediate precision was also performed within laboratory variation on different days for all three drugs simultaneously in five replicate at five concentrations.

Analyst- to analyst variation: Analyst to analyst variation was performed by different analyst in five replicate at five concentrations.

Reproducibility: The reproducibility was performed in different laboratory (SHIMAZDU 1800 series) in five replicate at five concentrations. Result of precision shown in Table 3.

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at five replicate and five concentrations level. The value of mean of recoveries was found to be in ranging from 98.77 to 99.06% for amlodipine, 98.89 to 99.34% for olmesartan and 98.68 to 98.96% for hydrochlorthiazide. The value of SD and %RSD are less then 2 indicate the accuracy of method. Result of recovery study shown in Table 3.

Robustness

Standard stock solution of 1000µg/ml of amlodipine, olmesartan and hydrochlorthiazide were prepared using 95% methanol as a solvent. From standard stock solution, sub stock solution of 100µg/ml of amlodipine, olmesartan and hydrochlorthiazide were prepared

S.NO.	PARAMETER			MEAN* ± SD*			% RSD*		
1.	ACCURACY(RECOVERY STUDY)								
	AML	OLM	HCZ	AML	OLM	HCZ	AML	OLM	HCZ
	80%	80%	80%	98.77±0.03	99.24±0.02	98.68±0.04	0.033	0.023	0.043
	100%	100%	100%	99.68±0.04	98.89±0.02	98.95±0.05	0.043	0.028	0.052
	120%	120%	120%	99.06±0.03	99.34±0.02	98.96±0.03	0.036	0.026	0.033
2.	PRICISION								
	a. Repeatability			99.9±0.09	96.27±0.02	94.08±0.02	0.496	1.142	1.140
	b. Intermediate precision			99.38±0.05	96.40±0.18	93.68±0.24	0.481	1.075	1.073
	c.Analyst to Analyst			99.04±0.04	96.33±0.13	93.42±0.14	0.392	0.898	0.986
	d. Reproducibility			98.82±0.03	98.18±0.10	98.08±0.06	0.342	0.871	0.593
3.	ROBUSTNESS			99.40±0.09	95.95±0.14	93.61±0.23	0.733	0.151	0.246

Table 3: Result of validation parameter.

separately. From these standard stock solutions of drugs, appropriate dilutions was prepared to get mixed standard solutions of all three drugs in 5:6:5 ratio (amlodipine, olmesartan and hydrochlorthiazide). Results of robustness shown in Table 3.

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