Development of Spectrophotometric Method for Quantitative Estimation of Amlodipine Besylate, Olmesartan Medoxomil and Hydrochlorothiazide 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 hydrochlorothiazide in tablet dosage form. The stock solutions were prepared in methanol. The λ_{max} for amlodipine besylate, olmesartan medoxomil and hydrochlorothiazide were 238.5nm, 256.5nm and 271.5nm respectively. The amlodipine besylate, olmesartan medoxomil and hydrochlorothiazide 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; Hydrochlorothiazide

Introduction

Amlodipine besylate (Figure 1a) [1] is 3-Ethyl 5-methyl (4RS)-2-[(2-aminooethoxy)-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 hypertension [5]. Olmesartan medoxomil (Figure 1b) [1] is [2,3-dihydroxy-2-butenyl-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 Hydrochlorothiazide (Figure 1c) [1] is 6-chloro-3,4-dihydro-2H-1,2,4-benzenothiadiazine-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 Hydrochlorothiazide 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 hydrochlorothiazide 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 hydrochlorothiazide 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 Hydrochlorothiazide (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 hydrochlorothiazide 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 hydrochlorothiazide.

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 studying the overlay spectra it was observed that amlodipine shows maximum absorbance at 238.5nm, olmesartan shows maximum absorbance at 256.5nm and hydrochlorothiazide shows peaks at 271.5nm and 322nm. It was observed that hydrochlorothiazide 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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Received June 08, 2011; Accepted June 30, 2011; Published July 02, 2011

Citation: Sharma HK, Jain N, Jain SK (2011) Development of Spectrophotometric Method for Quantitative Estimation of Amlodipine Besylate, Olmesartan Medoxomil and Hydrochlorothiazide in Tablet Dosage Form. Pharm Anal Acta 2:126. doi:10.4172/2153-2435.1000126

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The concentration of all three drugs in mixture can be calculated by using following eqns, 

\[ C_{\text{AML}} = A_1\left(\frac{a_1}{a_2} - \frac{a_2}{a_3}\right) + A_2\left(\frac{a_2}{a_3} - \frac{a_3}{a_1}\right) + A_3\left(\frac{a_3}{a_1} - \frac{a_1}{a_2}\right) \] 

(1), 

\[ C_{\text{OLM}} = A_1\left(\frac{a_1}{a_2} - \frac{a_2}{a_3}\right) + A_2\left(\frac{a_2}{a_3} - \frac{a_3}{a_1}\right) + A_3\left(\frac{a_3}{a_1} - \frac{a_1}{a_2}\right) \] 

(2), 

\[ C_{\text{HCZ}} = A_1\left(\frac{a_1}{a_2} - \frac{a_2}{a_3}\right) + A_2\left(\frac{a_2}{a_3} - \frac{a_3}{a_1}\right) + A_3\left(\frac{a_3}{a_1} - \frac{a_1}{a_2}\right) \] 

(3), 

Where, \( A_1, A_2 \) and \( A_3 \) are the absorbance values of mixture/tablet solution. \( a_1, a_2, a_3 \) are the absorptivities of amlodipine at 238.5nm, 256.5nm and 271.5nm respectively, \( a_{\text{OLM}}, a_{\text{HCZ}} \) and \( a_{\text{HCZ}} \) are absorptivities of olmesartan 238.5nm, 256.5nm and 271.5nm respectively.

\[ C_{\text{AML}}, C_{\text{OLM}} \text{ and } C_{\text{HCZ}} \] are concentration of amlodipine, olmesartan and hydrochlorthiazide respectively.

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

\[ a_1 = 0.0366 \quad a_2 = 0.0338 \quad a_3 = 0.0070 \]

\[ a_2 = 0.0127 \quad a_3 = 0.0483 \quad a_1 = 0.0329 \]

\[ a_3 = 0.0045 \quad y_1 = 0.033 \quad a_2 = 0.0275 \]

The equation are:

\[ C_{\text{OLM}} = A_1\left(0.0024763382\right) - A_2\left(0.002528319\right) + A_3\left(0.00080425\right) / \]

\[ C_{\text{HCZ}} = A_1\left(0.000763382\right) - A_2\left(0.002528319\right) + A_3\left(0.00080425\right) / \]
was repeated at 5 concentration and 3 replicate level. A stock solution of amlodipine, olmesartan and hydrochlorthiazide was prepared from standard solutions of 0.00009798424 mg/ml of amlodipine, 20µg/ml of olmesartan and 12.5µg/ml of hydrochlorthiazide. It was diluted to 50 ml of methanol by sonication for about 10 minutes. The volume was made up to 10 ml using methanol and filtered by Whatmann filter paper (no.41).

Preparation for analysis of tablet formulation

Twenty tablets were taken and their average weight was determined. They were 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 20µg and 12.5µg respectively, the ratio of all three drugs was 5:20:12.5. This was done in solution in 50 ml of methanol by sonication for about 10 minutes. The volume was made up to 10 ml using 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 was determined by repeating the procedure over a short interval time. 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.

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. Solvent used was 100% methanol and does not show any significant interference in the spectrophotometric assay of all three drugs.

Results and Discussion

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

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, olmesartan and hydrochlorthiazide respectively. Result of linearity study shown in Table 2.

Intermediate precision: Intermediate precision was also performed within laboratory variation on different days and analyst to analyst variation by different analyst. The reproducibility is expressed in laboratory to laboratory variation.

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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.

Table 1: Result of Tablet formulation.

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

| 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 (r²) | 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 3: Result of Recovery study.

| S. No | DRUG  | MEAN | S.D. | %COV | Std. Error |
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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.

Acknowledgments

The authors are thankful to Sun pharmaceuticals, Silvasa(GJ), Plathico pharma Ltd. Dewas and Matrix laboratory Mumbai providing gift samples along with there analytical reports.

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| S.NO. | PARAMETER | MEAN* ± SD* | % RSD* |
|-------|-----------|-------------|--------|
| 1.    | ACCURACY(RECOVERY STUDY) |             |        |
|       | AML  | OLM  | HCZ | AML | OLM | HCZ | AML | OLM | HCZ |
| 80%   | 98.77±0.03 | 99.24±0.02 | 98.68±0.04 | 0.033 | 0.023 | 0.043 |
| 100%  | 99.68±0.04 | 98.89±0.02 | 98.95±0.05 | 0.043 | 0.028 | 0.052 |
| 120%  | 99.06±0.03 | 99.34±0.02 | 98.96±0.03 | 0.036 | 0.026 | 0.033 |
| 2.    | PRECISION |             |        |
| 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 | 0.886 |
| 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.