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Development and validation of spectrophotometric method for determination of Olmesartan, amlodipine and hydrochlorothiazide in combined pharmaceutical dosage forms

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Abstract

A simple, accurate, precise, economical and reproducible method was developed for simultaneous estimation of olmesartan, amlodipine and hydrochlorothiazide in Combined Pharmaceutical Dosage Forms. The excipients in the commercial tablet preparation did not interfere with the assay. The λmax for olmesartan, amlodipine and hydrochlorothiazide were 252 nm, 360 nm and 271 nm respectively. At 360 nm, Amlodipine showed some absorbance while olmesartan and hydrochlorothiazide showed zero absorbance so that amlodipine was estimated at 360 nm. While at 252 nm and 271 nm olmesartan and hydrochlorothiazide were determine by simultaneous estimation method after eliminating the absorbent of Amlodipine at this wavelength. Linearity in concentration range of 4-28 µg/mL, for all three drugs with mean recovery of 99.7 ± 0.15, 99.2 ± 0.24 % and 99.4 ± 0.18 % for OLM, AML and HTZ, respectively. Validation of the proposed method was carried out for its accuracy, precision, and reproducibility according to ICH guidelines. Thus the present study gives an excellent method for the determination of all the three drugs in combined dosage formulation without their prior separation

Keywords: Amlodipine besylate, hydrochlorothiazide, Olmesartan, Spectrophotometric method

Introduction

Olmesartan medoxomil (OLM) is angiotensin II antagonist used as an anti-hypertensive and chemically[1] it 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.

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It works by blocking a substance in the body that causes blood vessels to tighten. As a result, This lowers blood pressure and increases the supply of blood and oxygen to the heart. Literature survey reveals various methods like RP-HPLC [2, 3, 4] Spectrophotometric [5, 6] difference spectrophotometric [7] methods for estimation of Olmesartan medoxomil single dosage form and combination with other drugs. Amlodipine besylate (AML) is a long-acting calcium channel blocker used as an anti-hypertensive in the treatment of angina and chemically[8] is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate It affects the movement of calcium into the cells of the heart and blood vessels. As a result, amlodipine relaxes blood vessels and increases the supply of blood and oxygen to the heart while reducing its workload. It is official in IP. IP describe HPLC[9] method for its estimation Literature survey also reveals that Spectrophotometric[10] HPLC[11] Spectrofluorometry [12] Differential-Pulse Voltammetry [13] LC[14], LC/MS/MS [15] and HPTLC[16] methods have been reported for the estimation of AML in pharmaceutical formulations and in biological fluids. Hydrochlorothiazide (HTZ) is thiazidet class of diuretics and chemically it [17] is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide. It reduces the amount of water in the body by increasing the flow of urine, which helps lower the blood pressure. It is official in IP, BP and USP. IP describe Spectrophotometric [18], BP and USP describe HPLC[19,20] method. Literature survey reveals various methods like uv-spectrophotometric method [21], RPHPLC [22], LC/MS/MS [23], HPTLC [24], diffuse reflectance spectroscopy [25] for estimation of Hydrochlorothiazide single dosage form and combination with other drugs in pharmaceutical dosage forms and in biological fluid. Olmesartan, Amlodipine and Hydrochlorothiazide combination is not official in any pharmacopoeia so no official method is available for estimation of these drugs in combined dosage form. Literature survey reveals that there is one reported stability indicating UPLC[26] method for the determination of olmesartan, amlodipine and hydrochlorothiazide in pharmaceutical dosage form. But no method has been found for the simultaneous estimation of Olmesartan, amlodipine and hydrochlorothiazide combinations by spectroscopy method. The present manuscript describes simple, sensitive, accurate, precise, reproducible, and economical Spectrophotometric method for simultaneous estimation of olmesartan, amlodipine and hydrochlorothiazide in combined dosage form.

Materials and methods

Apparatus

A Shimadzu (UV-1700) double beam UV-Visible spectrophotometer attached with computer operated software UV probe with spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, Sartorius CP224S analytical balance (shimadzu, zapan), ultra sonic cleaner (Life care eq. PVT. LTD, Mumbai, India) and volumetric flasks were used during the study.

Reagents and Materials

Pharmaceutical grade of olmesartan (OLM), Amlodipine besylate (AML) and Hydrochlorothiazide (HTZ) were kindly supplied as a gift samples from Torrent Pharmaceutical Ltd, Gujarat (India) with 99.97% purity. The pharmaceutical formulations containing 20 mg OLM, 5 mg AML and 12.5 mg HTZ of brand OLMAT –AMH tablet was procured from the local pharmacy. Methanol (AR grade) was purchased from Fisher scientific, Mumbai, India, Whatman filter paper no. 41 (Whatman International Ltd., England).

Analytical Conditions

Absorbance spectrum of pure OLM, AML and HTZ were scanned in the spectrum basic mode. By dilution of three standard drug solutions with methanol, solutions containing 10 µg ml^{-1} of OLM, 10 µg ml^{-1} of AML and 10 µg ml^{-1} of HTZ were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both all the drugs. OLM, AML and HTZ showed absorbance maxima at 252 nm, 360 nm and 271 nm respectively. The overlain spectra showed λmax of all drugs (Fig. 1). The arrangement of spectra of studied compound of favour is that amlodipine has absorbance maxima at 360 nm and at that wavelength, in the studied concentration range; the spectra of OLM and HTZ solution show no absorbance. So the concentration of Amlodipine was calculated from the absorbance measured at maxima at 360 nm. Then, after calculating the AML concentration in investigated sample, the absorbance was established in which AML is participating at the measured wavelength for OLM and HTZ, i.e. at 252 nm and 271 nm. The concentration of OLM and HTZ in mixtures was calculated according to simultaneous equation method after eliminating the absorbance of AML at this wavelength.

Preparation of OLM, AML and HTZ Standard Stock Solutions

Accurately weighed portions of OLM (10 mg), Amlodipine besylate (13.86 mg, which is equivalent to Amlodipine 10 mg) and HTZ (10 mg) were transferred to a separate 100 mL volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentrations of OLM (100 µg/mL), AML (100 µg/mL) and HTZ (100 µg/mL).

Preparation of Sample Solutions

Twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 20 mg of OLM, 5mg of AML and 12.5mg of HTZ was transferred into a 100 ml volumetric. Methanol (50 mL) was added to it and sonicated for 20 min. The solution was filtered through whatman filter paper No. 41 and the volume was adjusted up to the mark with Methanol. This solution is expected to contain 200 µg/mL OLM, 50 µg/mL AML and 125 µg/mL HTZ. From this solution 1.2 ml was taken in to a 10 mL volumetric flask and the volume was adjusted up to mark with Methanol to get a final concentration of OLM (16 µg/mL), AML (6 µg/mL) and HTZ (15 µg/ml).

Method Validation

(a) Calibration curves were plotted over a concentration range of 4-28 µg/mL for OLM, AML and HTZ. Accurately measured standard working solutions of OLM, AML and HTZ (0.4, 1.0, 1.6, 2.0, 2.4, and 2.8 mL) were transferred to a three series of 10 mL of volumetric flasks and diluted to the mark with methanol and absorbances were measured at 360 nm, 271 nm and 252 nm for three drugs. The calibration curves were constructed by plotting absorbance at 360 nm versus concentrations for AML and absorbance at 252 nm and 271 nm versus concentration for OLM and HTZ respectively.

(b) Accuracy (% Recovery)

The accuracy of the methods was determined by calculating recoveries of OLM, AML and HTZ by the standard addition method. Known amounts of standard solutions of OLM, AML and HTZ. (7, 10.5, 14 µg/mL forl OLM, AML and HTZ) were added to prequantified sample solutions of tablet dosage form. The amounts of OLM, AML and HTZ were estimated by applying obtained values (n=6) to the regression equation of the calibration curve.

(c) Method Precision (% Repeatability)

The precision of the instruments was checked by repeated scanning and measurement of absorbance of solution of (n = 6) of OLM, AML and HTZ (10 µg/mL) without changing the parameter.

(d) Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed method were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of OLM, AML and HTZ (10, 15, and 20 µg/ml for all). The results were reported in terms of relative standard deviation (% RSD).

(e) Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines [27].

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ= the standard deviation of the response and S = Slope of calibration curve.

(f) Analysis OLM, AML and HTZ in Combined Dosage Forms

Pharmaceutical formulation of OLM, AML and HTZ was purchased from local pharmacy. The responses of formulations were measured at 252 nm, 271 nm and 360 nm for quantification of OLM, AML and HTZ as described above. The amounts of OLM, AML and HTZ present in sample solution were determined by fitting the responses into the regression equation for OLM, AML and HTZ.

Results and discussion

Method development

An attempt has been made to develop a fast, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of OLM, AML and HTZ in their combined dosage form. In this method, the absorbance was measured at three wavelengths, one at 360 nm at that wavelength, no absorbance of

OLM and HTZ solution. So the concentration of Amlodipine was calculated from the absorbance measured at maxima at 360 nm.

Table 1. Specific absorbance of Amlodipine, Olmesartan and hydrochlorothiazide in methanol

NAME	$A^{1\%}_{1cm}$		
	252 nm	**271 nm**	**360 nm**
Amlodipine	110	30	80
Olmesartan	380	130	-
Hydrochlorothiazide	180	580	-

Then, after measured the absorbance of OLM and HTZ at 252 nm and 271 nm. The concentration of OLM and HTZ in mixtures was calculated according to simultaneous equation method after eliminating the absorbance of AML at this wavelength. For this measurement, the solutions of OLM, AML and HTZ were prepared separately in methanol. They were scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 1 nm. From the overlain spectra of the three drugs (Fig 1) absorbencies (Table 1) were measured at selected wavelength. The absorbance substituted in the following equations (1, 2, and 3) to obtain the concentration OLM, HTZ and AML in g/100 ml respectively.

$$C_x = \frac{(A1*580) - (A2*180)}{197000} \quad \text{..... (1)}$$

$$C_y = \frac{(A1*130) - (A2*380)}{197000} \quad \text{..... (2)}$$

$$Cz = \frac{A3 + 0.002}{0.011} \quad \text{..... (3)}$$

Where,

C_X, C_Y and Cz = the concentration of OLM, HTZ and AML respectively.

A1 = the absorbance of mixture at 252 nm

A2 = the absorbance of mixture at 271 nm

A3 = the absorbance of mixture at 360 nm

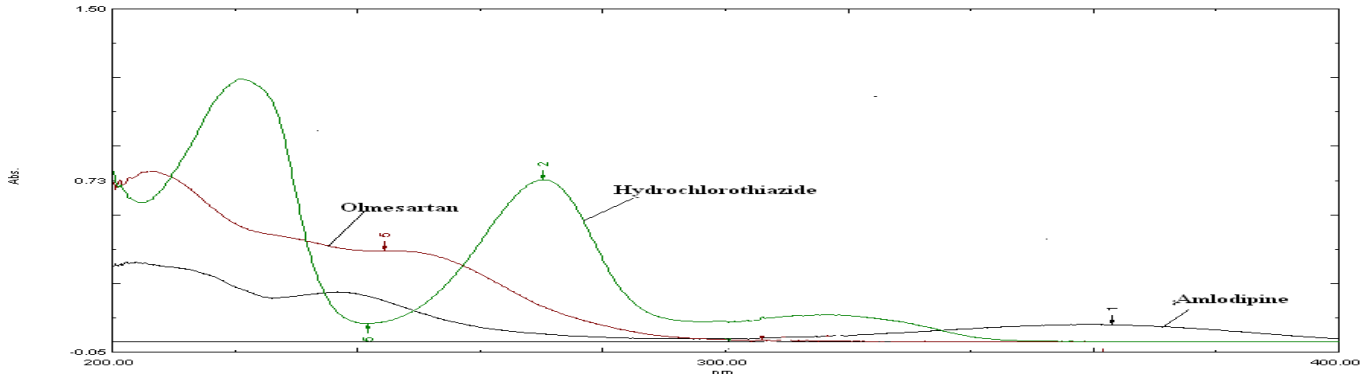

Figure 1 Overlain absorption spectra of Standard solutions of Olmesartan, Amlodipine and Hydrochlorothiazide in methanol

Validation of the Proposed Method

Linearity - Linear correlation was obtained between absorbance Vs concentrations of OLM, AML and HTZ in range of 04-28 µg/mL. The linearity of the calibration curves was validated by the high value of correlation coefficients of regression (Table 2).

Table 2. Regression analysis of calibration curve for OLM, AML and HTZ for the Spectrophotometric method

Parameters	**Spectrophotometric method**		
	OLM	AML	HTZ
Linearity range(µg/mL)	4-28	4-28	4-28
Slope	0.0255	0.0073	0.0280
Standard deviation of slope	0.0101	0.0040	0.0096
Intercept	0.037	0.0016	0.0067
Standard deviation of intercept	0.0062	0.0015	0.0072
Correlation coefficient, *r*	0.9977	0.9998	0.9957

Accuracy - The recovery experiments were carried out by the standard addition method. The mean recoveries OF OLM, AML and HTZ was obtained 101.7 ± 0.23 % , 99.7±0.75 and 99.2 ± 1.57 % for ±, respectively (Table 3,4). The high values indicate that method is accurate.

Table 3. Summary of validation parameters for the spectrophotometric method.

Parameters	**Spectrophotometric Method**		
	OLM	AML	HTZ
LODa, µg/mL	0.61	0.32	1.09
LOQb, µg/mL	1.85	0.97	3.3
Accuracy, % (*n* = 3)	101.7 ± 0.23	99.7±0.75	99.2 ± 1.57 %
Repeatability, % RSDc (*n*=6)	0.24	1.13	1.46
Precision, % RSD			
Interday (*n* = 6)	0.45-1.26	0.23-1.13	0.27-1.63
Intraday (*n* = 6)	0.39-1.81 %	0.73-1.84	0.27-1.63

aLOD = Limit of detection, bLOQ = Limit of quantification

cRSD = Relative standard deviation, *n* = number of determinations

Table 4. Results of recovery study for OLM, AML and HTZ by the Spectrophotometric method (n=3)

Method	Drug	Amount of sample taken (µg/mL)	Amount of standard spiked (%)	Mean % Recovery± SDa*
Spectrophotometric method	OLM	14 14 14	50 75 100	99.41 ± 1.79 98.71 ± 1.41 100.82 ± 1.05
	AML	14 14 14	50 75 100	99.59 ± 1.27 99.31 ± 1.21 98.95 ± 1.01
	HTZ	14 14 14	50 75 100	98.99±0.98 99.36±1.02 98.97±1.25

a SD = Standard deviation
n = Number of determinations

Method precision - The % RSD values for OLM, AML and HTZ were found to be 0.24, 1.13 and 1.46 (Table 3). The low values of RSD indicate the proposed method is repeatable.

Intermediate precision - The low RSD values of interday (0.45-1.26 %, 0.23-1.13 % and 0.27-1.63 %) and intraday (0.39-1.81 %, 0.73-1.84 % and 0.27-1.63 %) variations for OLM, AML and HTZ, respectively reveal that the proposed method is precise (Table 3).

LOD and LOQ - LOD for OLM, AML and HTZ were found to be 1.45 µg/mL, 0.43 µg/mL and 1.09 µg/mL, respectively. LOQ for OLM, AML and HTZ were found to 1.85 µg/mL, 0.97 µg/mL and 3.3 µg/mL, respectively (Table 2). These data show that the method is sensitive for the determination of OLM, AML and HTZ.

Assay of the pharmaceutical formulation

The proposed validated method was successfully applied to determine OLM, AML and HTZ in their combined dosage form .The spectra of sample is shown in figure 2. The results obtained for OLM, AML and HTZ were comparable with the corresponding labeled amounts (Table 5).

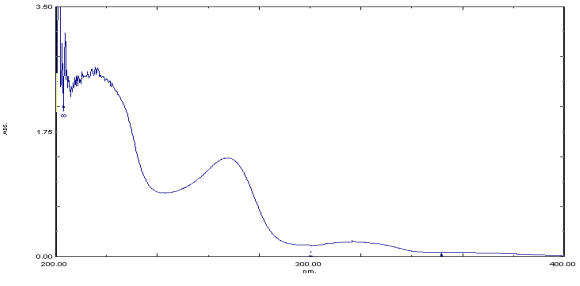

Figure 2 Absorption Spectra of sample solution of Olmesartan, Amlodipine and Hydrochlorothiazide in methanol

Table 5. Assay results for the combined dosage form using the proposed Spectrophotometric (*n* = 6) method.

Parameters	Spectrophotometric method		
Brand A	OLM ± SDa	AML ± SDa	HTZ ± SDa
	99.80 ± 1.21	99.41 ± 1.01	99.89 ± 1.75

aSD = Standard deviation,*n* = Number of determinations

Conclusion

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and is in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of OLM, AML and HTZ. The method can be routinely used for the analysis of the OLM, AML and HTZ in combined dosage form.

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