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Full Length Original Research Paper

Two novel spectrophotometric methods for determination of ternary mixture used as antihypertensive therapy in combined tablet dosage formulation

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Abstract:
Aim: To develop and validate two novel spectrophotometric methods for the simultaneous determination of ternary mixture of Olmesartan medoxomil, Amlodipine besylate and Hydrochlorothiazide.
Methods: The proposed methods, successive ratio derivative method and double divisor method involved treatment of normal absorption spectra of ternary mixture in UV probe software for the simultaneous determination of Olmesartan medoxomil, Amlodipine besylate and Hydrochlorothiazide in bulk and tablet dosage formulation without prior separation.
Results: All the drugs exhibited good linearity over the reported concentration range with acceptable correlation coefficient. The method was validated according to ICH guidelines for evaluation of accuracy, repeatability, reproducibility, sensitivity showing acceptable percent relative standard deviation of less than 2.
Conclusion: The proposed methods demonstrated that these are simple, rapid, accurate and precise methods and can be used for routine analysis of bulk and tablet dosage formulation in quality control laboratories eliminating the need of prior separation of the pharmaceutical mixtures.

Keywords: successive ratio derivative method, double divisor method, Olmesartan medoxomil, Amlodipine besylate, Hydrochlorothiazide, UV spectrophotometry.

1. Introduction

Cardiovascular diseases are the disorders of heart and blood vessels and primarily include coronary heart disease, hypertension, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. Cardiovascular diseases are the major cause of death in developed countries, projecting almost 1.5 billion people by 2025[1]. The risk of cardiovascular morbidity and mortality is directly correlated with blood pressure. More than 90 percent of patients have essential hypertension, a disorder of unknown origin affecting the blood pressure regulating mechanism and continues to be one of the most significant risk factors for the development of cardiovascular disease worldwide. Diuretics, β-blockers, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, and calcium channel blockers are considered primary antihypertensive agents. These agents, either alone or in combination, are used to treat the majority of hypertensives[2-3]. One of the widely used and available antihypertensive therapy is triple combination of olmesartan medoxomil, amlodipine besylate and hydrochlorthiazide.

Olmesartan medoxomil (OLM), a non- peptide molecule, is an angiotensin II receptor (type AT1) antagonist and chemically is 5-methyl-2-oxo-2H-1,3-dioxol-4-yl) methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl) phenyl) phenyl}methyl-1H-imidazole-5-carboxylate[2,4].

OLM has not yet been officially described in any

pharmacopoeia. Hydrochlorothiazide (HCTZ), a thiazide diuretic, is chemically 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. Amlodipine besylate (AMLO), a beta-blocker, chemically is, 4-[2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzene acetamide[2, 3, 5]. Official methods have been reported individually for AMLO[6,7] and HCTZ[6-8]. Literature reviewed reports several nonofficial analytical methods including HPLC[9-11], a stability-indicating HPLC[12-16], HPTLC[17,18] and UV-spectrophotometry by simultaneous equation method[19-21] for the determination of OLM, AMLO and HCTZ either single or in combination with other drugs. However, the literature on the development of successive ratio derivative and double divisor UV spectrophotometric method for simultaneous estimation of OLM, AMLO and HCTZ in the combined dosage form is lacking.

The analytical method development using sophisticated instruments such as spectrophotometer, HPLC, GC and HPTLC needs a prior separation or extraction procedure, for the analysis of multi-mixtures. These analytical methods are easy to perform, precise and show reproducible results as compared to any other methods. One of the most exploited method for analysis of drugs is spectrophotometry. Simultaneous quantitative analysis of pharmaceuticals containing more than one active compound is difficult to perform by classical spectrophotometric method due to overlapping spectra[22]. Additionally, several sophisticated instrumental techniques based on separation like HPLC, capillary electrophoresis etc. allow the separation and determination of the content of samples; however these techniques bring high cost and time consumption. One way of elimination of these drawbacks is application of numerical and graphical techniques to original absorption spectra. The use of spectrophotometric techniques combined with mathematical algorithms and wavelength transformation has brought a new, fast and easy to apply methodology for the determination of analytes in complex samples without prior separation[23-28]. In context to this, this research paper focusses on the development of successive ratio derivative and double divisor method for the analysis of ternary mixture of OLM, AMLO and HCTZ in bulk and tablet formulation.

2. Material and methods

2.1 Chemicals and materials

Working standards of OLM, AMLO and HCTZ were kindly provided as a gratis sample from Glenmark Generics Limited, Pune; Prudence Pharma Chem, Ankleshwar and Ipca Laboratories Limited, Ratlam respectively. All solvents and chemicals used were of analytical grade, purchased from Merck Specialities Pvt. Ltd., India. Marketed tablet formulations used in this study were procured from local market; Triolmezest film-coated tablet, from Sun Pharmaceutical Industries Ltd. was used in this study.

2.2. Instrumentation

A double beam UV-Visible spectrophotometer (Shimadzu, Japan) model UV-1800 with a quartz cell of 1 cm path length and fixed slit width (2nm), UV probe software, Shimadzu version 2.34; Electronic analytical balance (AUX-220), Shimadzu and Ultrasonic cleaner Toshniwal instrument Pvt. Ltd. were used in the study.

2.3. Procedure

2.3.1. Preparation of stock and calibration standard solutions of OLM, AMLO and HCTZ

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Accurately weighed quantity of OLM, AMLO and HCTZ (10 mg) were transferred into 100 ml volumetric flask, dissolved and diluted up to the mark with methanol to prepare a stock solution having a final concentration of 100 µg/ml for OLM, AMLO and HCTZ. From this standard stock solution, appropriate aliquots were diluted up to 10 ml with methanol to prepare final concentration in the range of 4-20 µg/ml for OLM, AMLO and HCTZ.

2.3.2. Successive ratio derivative method

The Zero-order (D_0) absorption spectra for mixture of OLM, AMLO and HCTZ in the final concentration range were recorded by scanning in the range of 200-400 nm and stored in computer. For calibration curve of OLM, the zero order spectra of the solutions of different concentration of mixture recorded in the calibration range were divided by standard spectrum of AMLO (10 µg/ml) to record first ratio spectra and then converted to first order derivative. Further, the stored spectra of standard solution of HCTZ was divided by standard solution of AMLO to record ratio spectra that was further converted mathematically to first order derivative. Finally, both the outputs in the form of first order derivative were further divided to record second ratio spectra and the output was further transformed to first order derivative. The minimum or maximum of the first derivative second ratio spectra with respect to wavelength was used for the construction of calibration graph for OLM. Similar procedure was used for the other two components; AMLO and HCTZ by using divisor concentration of 10 µg/ml for each drug.

2.3.3. Double divisor ratio spectra method

For determination of OLM, the stored spectra of the solutions of ternary mixture were divided by sum of standard spectrum of AMLO (10 µg/ml) and HCTZ (10 µg/ml) to record ratio spectra, followed by first derivative transformation of these vectors with respect to wavelength. The minimum or maximum of the first derivative of these ratio spectra with respect to wavelength was recorded for the construction of calibration curve. Similar procedure was followed for the other two components; AMLO and HCTZ by using divisor concentration of 10 µg/ml for each drug.

2.4. Validation of method

Both the methods were validated in accordance with ICH guidelines Q2 (R1) for evaluation of various parameters; linearity, precision, accuracy, limit of detection, limit of quantification and specificity[29]. Linear relationship between absorbance and concentration of all three drugs were evaluated over the concentration range of 4-20 µg/ml for OLM, AMLO and HCTZ respectively by making five replicate measurements. Calibration plots were constructed by plotting the absorbance versus the concentration and treated using the method of ordinary least squares regression analysis. As per ICH guideline, limit of detection and quantitation of the developed method were calculated from the standard deviation of the response and slope of the calibration curve of each drug using the formula, Limit of detection=3.3xσ/S, Limit of quantification=10xσ/S, where, "σ" is standard deviation of response, "S" is slope of calibration curve. Precision of the developed method was evaluated by performing repeatability on same day and intermediate precision studies on different days in three replicates. Repeatability and intermediate precision was performed for three different concentrations (8, 12 and 16 µg/ml for OLM, AMLO and HCTZ) and absorbance measured was expressed in terms of percent relative standard deviation (% R.S.D.). Accuracy of method was ascertained by performing recovery

study by standard addition method at three concentration levels (50%, 100% and 150%) in triplicate. Recovery studies for OLM and HCTZ were carried out by spiking three different concentrations of OLM and HCTZ standard (3 µg/ml, 6 µg/ml and 9 µg/ml) to the formulation (6 µg/ml). Similarly, recovery studies for AMLO were carried out by spiking three different concentrations of AMLO standard (2.5 µg/ml, 5 µg/ml and 7.5 µg/ml) to the formulation (5 µg/ml).

2.5. Analysis of marketed formulation

The proposed methods were used for simultaneous estimation of OLM, AMLO and HCTZ in tablet dosage formulation. Twenty tablets were ground in fine powder form and powder equivalent to 20.00 mg of OLM, 12.50 mg of HCTZ and 5.00 mg of AMLO accurately weighed was transferred in 50 ml volumetric flask. About 15 ml of methanol was added and the mixture was sonicated for 15 minutes. The mixture then was diluted to volume with methanol, mixed well and filtered to obtain the sample stock solution. For the determination, 5 ml of stock solution was diluted to volume 10 ml with methanol to obtain a final concentration 20 µg/ml of OLM, 5 µg/ml of AMLO and 12.5 µg/ml of HCTZ. Procedure similar to standard mixture was followed in UV probe software for recording and scanning of the spectra of OLM, AMLO and HCTZ and repeating analysis in triplicate.

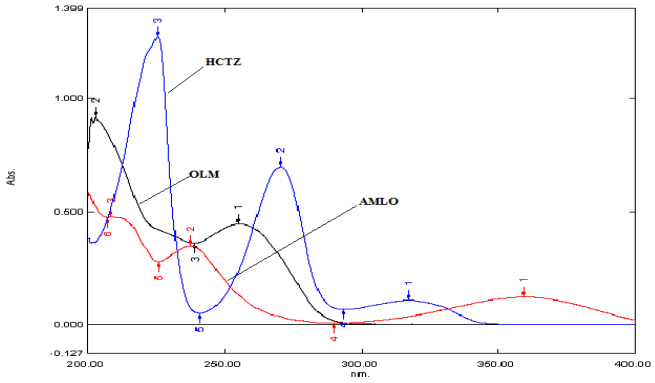
2.6. Statistical analysis

Statistical parameters like SD, %RSD were computed by using MS Excel.

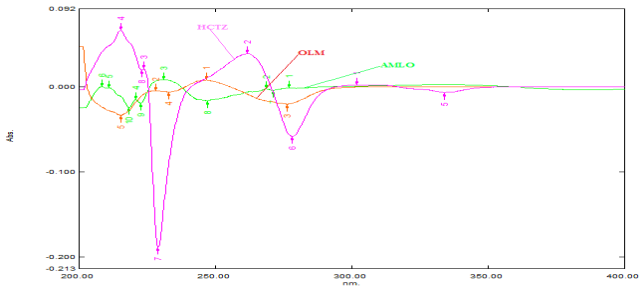
3. Results and Discussion

3.1. Development and Optimization of method

Zero-order absorption (D_0) overlay spectra of OLM, AMLO and HCTZ (Fig. 1a) shows considerable overlapping of bands and therefore, zero order absorption spectra was transformed to first order and second order derivative spectra. Both first order and second order overlay spectra of OLM, AMLO and HCTZ (Fig. 1b and 1c) shows interference of other drug at zero-cross over point (ZCPs) of each selected drug, revealing that their simultaneous determination is difficult in their combined dosage form by first order and second order derivative spectrophotometric method. In context to this, as described above, two methods successive ratio derivative (method I) and double divisor method (method II) are proposed. The chosen divisor concentration (10 µg/ml) gave good results for the slope, intercept and correlation coefficient of calibration graphs. The wavelength selected for estimation of OLM (267 nm), AMLO (242.6 nm) and HCTZ (219 nm) gave good correlation coefficient for method I (Fig. 2a - c). Similarly, acceptable correlation coefficient was obtained for the wavelength selected for estimation of OLM (232.18 nm), AMLO (339.59 nm) and HCTZ (228.99 nm) using method II respectively (Fig. 3a-c).



(a)



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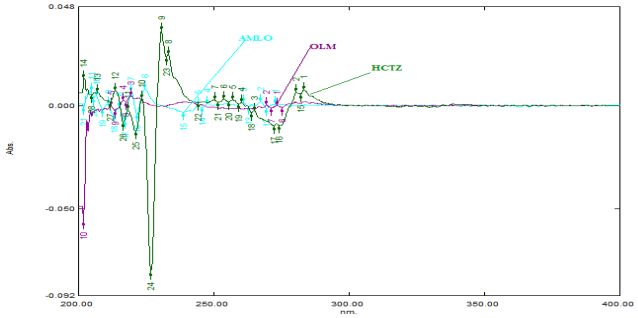
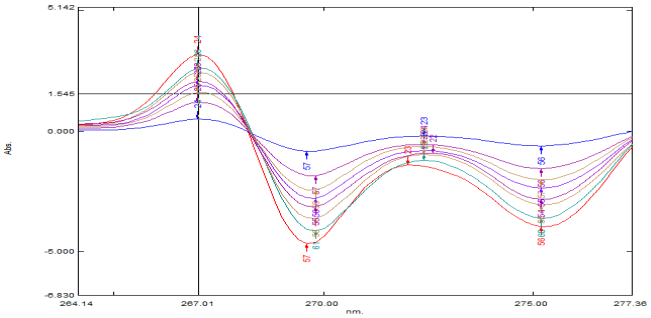


(c)

Fig. 1 a-c: Overlay spectra of OLM (10 µg/ml), AMLO (10 µg/ml) and HCTZ (10 µg/ml), (a) Zero-order (D_0); (b) First-order derivative; (c) Second-order derivative



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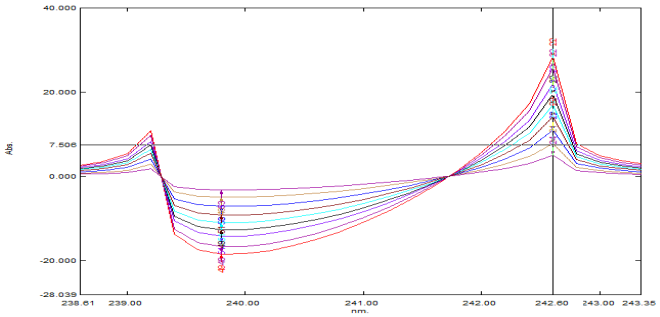

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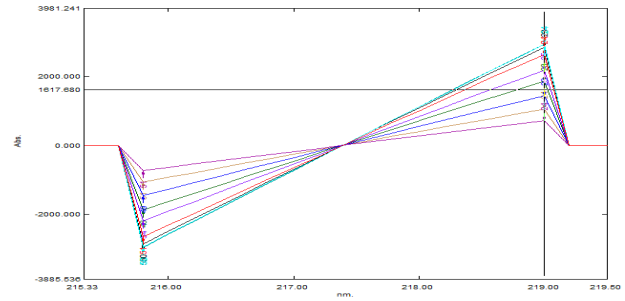
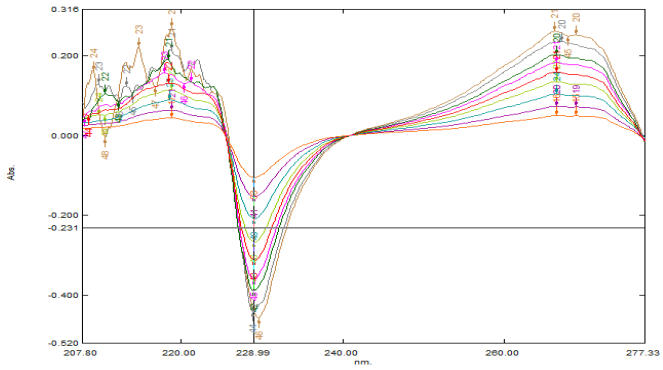


(c)

Fig. 2 a-c: (a) First order derivative (D_1) of second ratio spectrum of OLM (4-20 µg/ml); (b) First order derivative (D_1) of second ratio spectrum of AMLO (4-20 µg/ml); (c) First order derivative (D_1) of second ratio spectrum of HCTZ (4-20 µg/ml)



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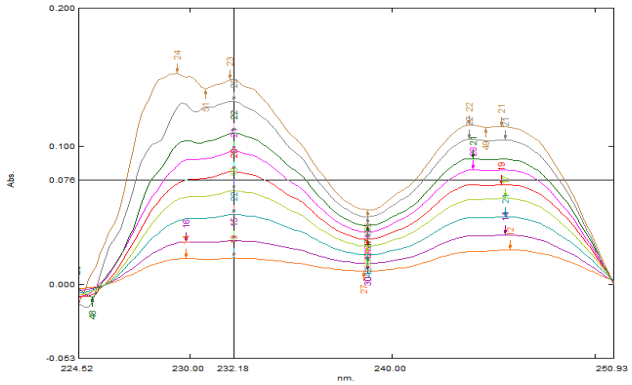

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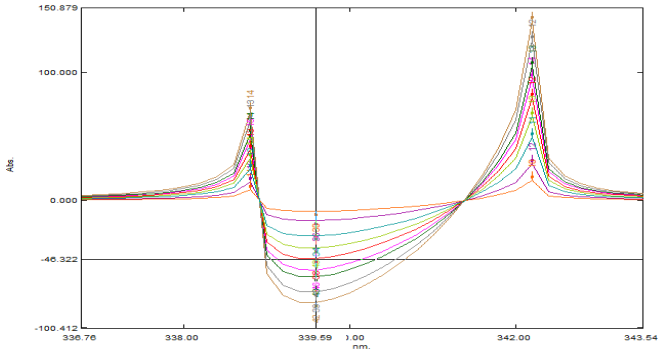

(c)

Fig. 3 a-c: (a) First-order derivative (D_1) Ratio spectra of HCTZ (4-20 µg/ml); (b) First order derivative (D_1) ratio spectra of OLM (4-20 µg/ml); (c) First order derivative (D_1) ratio spectra of AMLO (4-20 µg/ml)

3.2. Validation of method

Method validation was performed according to the ICH guidelines for proposed methods. Linearity of the proposed methods was evaluated and good linearity is evident from the high value of the

correlation coefficient (Table 1). The limit of detection and limit of quantification of developed method shows high sensitivity of both the methods (Table 1). Precision of the proposed methods in terms of repeatability and intermediate precision was evaluated at three concentrations of 8, 12 and 16 µg/ml and percent RSD was found to be less than 2 indicating reproducibility of both the developed methods (Table 2). Accuracy further assessed by the standard addition method at three concentration levels in tablet formulation, showed mean percentage recovery at all three levels in the range of 98.26% to 102.99%, suggesting suitability of method to perform routine drug analysis (Table 3). Specificity of the proposed methods is evident from the spectra shown in Fig. 3 and 4. Percentage amount found for all the three drugs from marketed formulation were within the range of 99.94-100.85% and 99.66-101.49% for method I and II, revealing no interference from excipients and good accuracy of the proposed methods (Table 4).

Table 1: Linear regression parameters for HCTZ, AMLO and OLM by both proposed methods

Parameter	HCTZ[a]		AMLO[a]		OLM[a]	
	METHOD I	METHOD II	METHOD I	METHOD II	METHOD I	METHOD II
Wavelength (nm)	219	228.99	242.6	339.59	267	232.18
Calibration range(µg/ml)	4-20	4-20	4-20	4-20	4-20	4-20
Correlation coefficient (r^2)	0.9932	0.9930	0.9980	0.9966	0.9953	0.9955
Slope ± SD[a](S_a)	1.1225	0.0005	0.0046	0.0022	0.0008	0.0001
Intercept ±SD[a] (S_b)	7.3160	0.0038	0.0657	0.0158	0.0068	0.0008
Limit of detection (µg/ml)	0.125	0.484	0.146	0.011	0.136	0.348
Limit of Quantitation (µg/ml)	0.379	1.467	0.444	0.034	0.412	1.055

[a] Average of five determinations

Table 2: Recovery study at three concentration levels for HCTZ, AMLO and OLM by both proposed methods.

Drug	Concentration of standard added	Method	Mean % Recovery[a]	%RSD
OLM	50%	I	101.53	1.48
		II	100.18	1.25
	100%	I	99.32	1.41
		II	102.99	1.57
	150%	I	102.53	1.01
		II	102.08	1.72
AMLO	50%	I	98.26	1.37
		II	100.47	0.57
	100%	I	98.93	0.94
		II	102.23	0.24
	150%	I	99.01	0.97
		II	100.69	0.23
HCTZ	50%	I	99.4	0.80
		II	101.5	1.18
	100%	I	100.04	1.07
		II	100.23	1.08
	150%	I	101.96	0.77
		II	99.93	1.57

[a] mean of three determinations at three concentration level of standard (for OLM and HCTZ; 3µg/ml, 6µg/ml and 9µg/ml, for AMLO; 2.5µg/ml, 5µg/ml and 7.5µg/ml); RSD=relative standard deviation

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Table 3: Precision study for HCTZ, AMLO and OLM by proposed method I and II

Drug	Repeatability[a] (% RSD)		Intermediate precision (% RSD)	
	Method I	Method II	Method I	Method II
HCTZ	0.0019-0.3517	0.741-1.293	0.0205-1.0200	0.730-1.804
AMLO	0.0512-0.2592	0.0076-0.048	0.1607-0.8336	0.028-0.378
OLM	0.4806-1.8217	1.6051-1.81	0.6998-1.7213	1.072-1.666

[a] average of three determinations for each concentration

Table 4: Analysis of HCTZ, AMLO and OLM in marketed formulation by proposed method I and II

Drug	Label claim(mg)	Mean %Assay [a]		%RSD	
	Method I and II	Method I	Method II	Method I	Method II
HCTZ	12.50mg	100.85	101.34	0.86	1.427
AMLO	5.00mg	99.94	99.66	1.14	0.368
OLM	20.00mg	100.26	101.49	1.38	1.235

[a] average of three determinations, RSD is relative standard deviation

4. Conclusion

Two novel and simple spectrophotometric methods, successive ratio derivative and double divisor ratio derivative were developed for the determination of OLM, AMLO and HCTZ in ternary mixture and tablet formulation using methanol as a solvent without prior separation. The validation of proposed methods according to ICH guideline proved that the method is simple, precise, reliable, sensitive and accurate. Moreover, when statistical T test was performed for comparing HPLC method[30] with the proposed methods; T test revealed that calculated values were less, HCTZ (1.35), AMLO (1.88), OLM (1.92) than the tabulated value (2.776 at 95% confidence interval), indicating no significant difference between the reported method and method I for analysis of marketed formulation. Similarly, calculated T values for method II for determination of marketed formulation were less, HCTZ (2.60), AMLO (2.41), OLM (1.92) than the tabulated value (2.776 at 95% confidence interval); thus indicating good accuracy of the proposed methods. These validated method showed good recovery for all the three drugs and hence can be used in the routine quality control for simultaneous estimation of mentioned drugs in ternary mixture and pharmaceutical formulation.

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