International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.5, pp 1518-1525, Sept-Oct 2014

Development and Validation of Double Divisor-Ratio Spectra Derivative Spectrophotometric Method for Simultaneous estimation of Olmesartan Medoxomil, Amlodipine Besylate and Hydrochlorthiazide in Tablet Dosage Form.

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Abstract: Double divisor-ratio spectra derivative method based on the Spectrophotometric data was developed for the simultaneous analysis of a ternary mixture containing Olmesartan Medoxomil (OLM), Amlodipine Besylate (AML) and Hydrochlorothiazide (HCTZ) in Methanol and Distilled water (70:30). The Double divisor ratio spectra derivative absorption minima at 258.98 nm was used for quantification of OLM, absorption maxima at 348.95 nm for quantification of AML and absorption maxima at 267.96 nm for quantification of HCTZ. The linearity was established over the concentration range of 4-20µg/ml, 4-20µg/ml and 3-15µg/ml for OLM, AML and HCTZ with correlation coefficient (r^2) of 0.9992, 0.9980 & 0.9976 respectively. The mean % recoveries were found to be in the range of 101.98 – 101.07%, 101.35 – 101.75 % and 99.02 – 100.80 % for OLM, AML and HCTZ respectively. The method is successfully applied to pharmaceutical formulation, with no interference from excipients as indicated by the recovery study. All validation parameters were within the acceptable range. The proposed method has been validated as per ICH guideline and successfully applied to the simultaneous estimation of OLM, AML, and HCTZ in their combined Tablet dosage form. The proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific.
Keywords: Olmesartan Medoxomil, Amlodipine Besylate, Hydrochlorothiazide, Double divisor-ratio spectra derivative spectroscopy.

Introduction

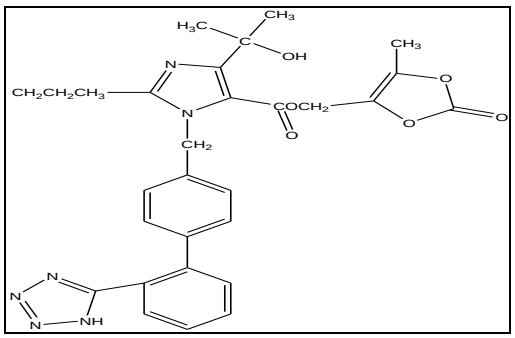
Olmesartan Medoxomil [1, 4] (OLM) is a prodrug and hydrolyzed to Olmesartan during absorption from the gastrointestinal tract. OLM is a selective AT1 subtype angiotensin II receptor antagonist. OLM (Fig. 1a) is described chemically as (5-methyl-2-oxo-1, 3-dioxol-4-yl) methyl ester of 4-(1-hydroxy-1-methylethyl) -2-propyl-1-{[20-(1H-tetrazol-5-yl) [1, 10-biphenyl]-4-yl] methyl}-1H-imidazole-5-carboxylic acid. Amlodipine Besylate [2, 4] (AML) is (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. Amlodipine Besylate (Fig. 1b) relaxes blood vessels and increases supply of blood and oxygen to heart while reducing its workload. Hydrochlorthiazide [3, 4] (HCTZ) is 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide. Hydrochlorthiazide (Fig 1c) reduces amount of water in body by increasing flow of urine, which helps lower blood pressure. It is a fixed dose triple combination with the potential to simplify dosing regimens, reduce pill burden and reduce costs. [1]

For the simultaneous determination of two or more active compounds in the same mixtures without a separation step, several spectrophotometric methods, such as classical derivative spectrophotometry [10–13],

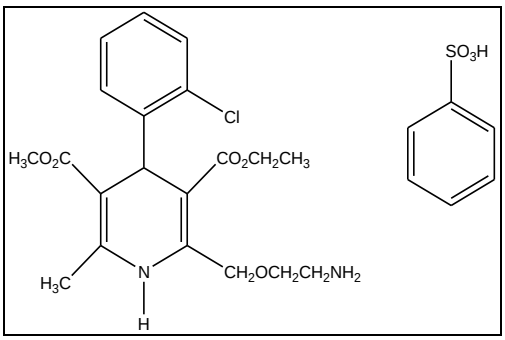
Vierordt's method [5] and its modified version [6], dual wavelength spectrophotometry [7–9] and some Chromatographic methods HPLC [14] , HPTLC [15] has been reported.

In this work, a new spectrophotometric method (double divisor ratio spectra derivative) has been successfully applied for the simultaneous determination of OLM, AML, and HCTZ in ternary mixture. Salinas et al. proposed the spectrophotometric method termed ratio-derivative spectrophotometry, for the simultaneous determination of two compounds in binary mixtures. Their method is based on the derivative of the ratio spectra for a binary mixture. The absorption spectrum of the mixture is divided by the absorption spectrum of a standard solution of one of the compounds and the first derivative of the ratio spectrum is obtained. The concentration of active compounds are then determined from the calibration graphs obtained by measuring the amplitudes at points corresponding to the minimum or maximum wavelengths. [16]

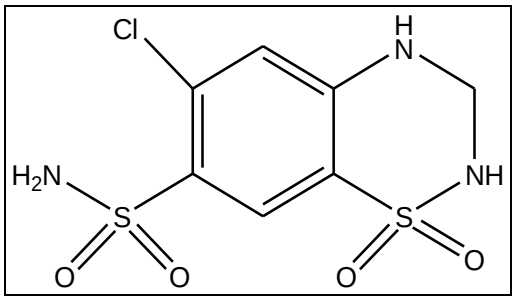
Recently, Dinc et al. has proposed a new spectrophotometric method for the simultaneous determination of ternary mixtures. This method is called "the double divisor ratio spectra derivative method". This method is based on the use of the coincident spectra of the derivative of the ratio spectra obtained by using a "double divisor" (sum of two spectra) and measuring at either the maximum or minimum wavelengths. [17, 18]



(a) Olmesartan Medoxomil



(b) Amlodipine Besylate



(c) Hydrochlorthiazide

Fig. 1: Chemical structures of Analysis

Experimental

Reagents and Chemicals

Olmesartan Medoxomil, Amlodipine Besylate and Hydrochlorthiazide were provided by Torrent Pharmaceutical Ltd., Ahmadabad (Gujarat, India). Methanol of AR grade was supplied from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Double-distilled water was procured from Sardar Patel University, Vallabh Vidyanagar.

Instruments and Apparatus

The Spectrophotometer used for study is Shimadzu-UV 1800 Spectrophotometer with wavelength accuracy (± 0.3 nm), 1 cm matched quartz cells and UV probe 2.35 software was used for all spectral measurements. Calibrated analytical balance Denver SI234,Germany was used for weighing purpose. Volumetric flasks, pipettes of borosilicate glasses were used in the study. All statistical calculations were carried out using Microsoft excel 2010 analytical tool.

Preparation of Standard Solutions

Accurately weighed 10 mg of OLM, AML and HCTZ standard were transferred separate 10 ml volumetric flask and dissolved in 5 ml methanol. The flasks were shaken and volume was made up to mark with diluents (Methanol: Distilled Water 70:30) to give solutions containing 1000 µg/ml OLM, AML and HCTZ. From this stock solutions, for OLM and AML 1 ml aliquots were transferred into two different 25 ml volumetric flasks and for HCTZ 1.5 ml aliquot was transferred in 50 ml volumetric flask. They were diluted up to mark with diluents to get working standard solution containing concentration of OLM, AML and HCTZ of 40, 40 and 30 µg/ml.

Selection of Analytical Wavelength

Solutions of OLM, AML and HCTZ were prepared in diluent by appropriate dilution and spectrum was recorded between 200-400 nm. The absorption spectra of the solutions prepared at different concentrations of OLM (4-20 µg/ml), AML (4-20 µg/ml) and HCTZ (3-15 µg/ml) were recorded and divided by the sum of the absorption spectra of solutions of AML+HCTZ, OLM+HCTZ, OLM+AML (10 µg/ml each in diluents) respectively as "double divisor" to get the ratio spectra and their first derivatives were plotted with delta lambda 10 nm and scaling factor 1.0. The divided and derivatized spectras (shown in Fig. 2,3 and 4) showed maximum and minimum wavelengths. The wavelength 258.98 nm, 348.95 nm and 267.96 nm were selected for analysis of OLM, AML and HCTZ respectively.

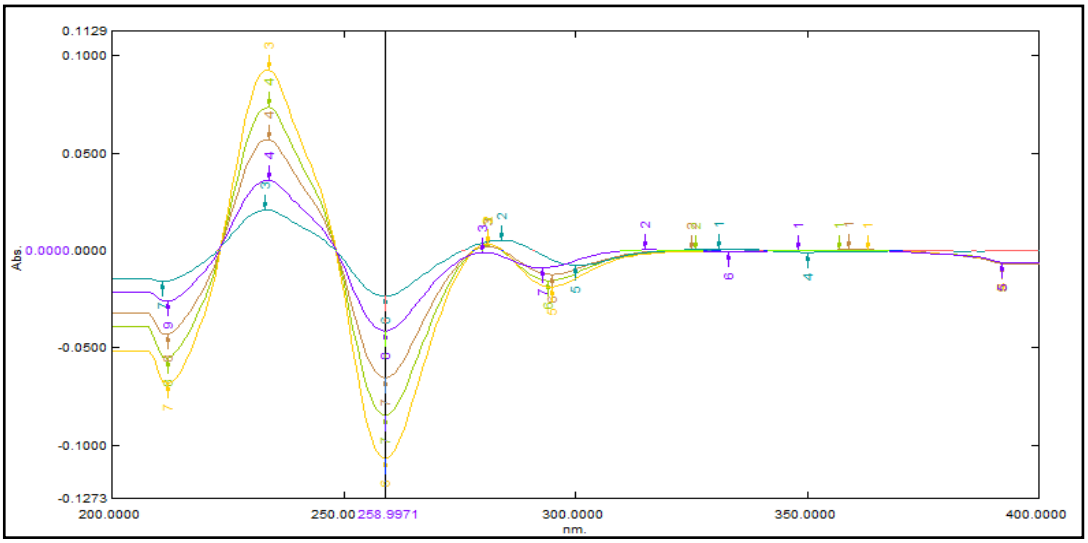


Fig. 2: Overlay First derivative Ratio Spectra of OLM at 258.97 nm (AML 10 µg/ml + HCTZ 10 µg/ml used as double divisor)

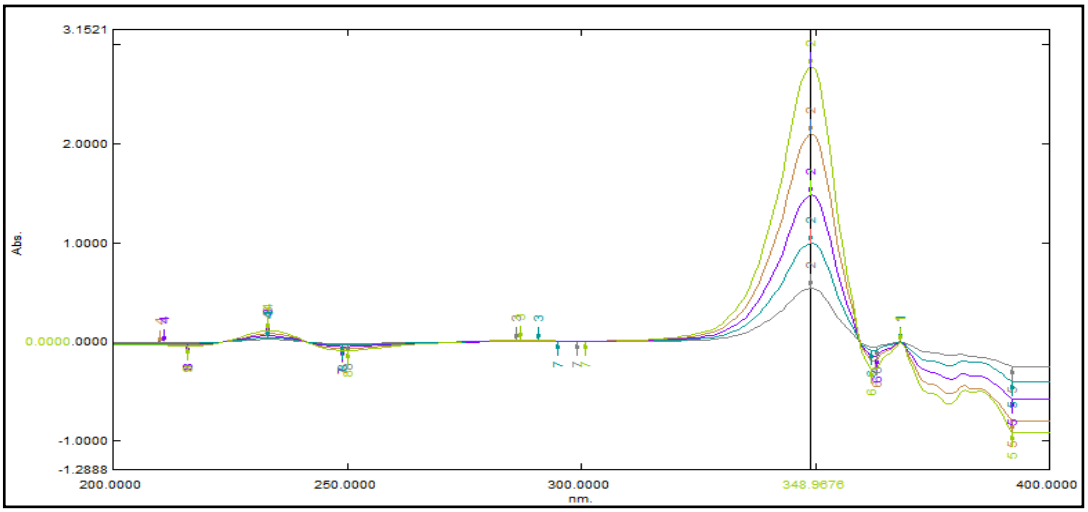


Fig. 3: Overlay First derivative Ratio Spectra of AML at 348.96 nm (OLM 10 µg/ml + HCTZ 10 µg/ml used as double divisor)

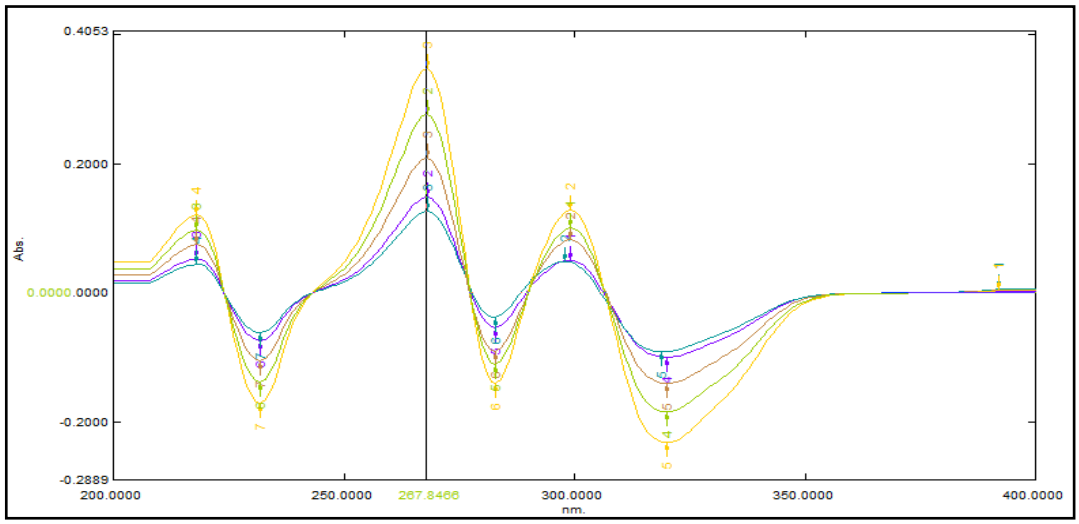


Fig. 4: Overlay First derivative Ratio Spectra of HCTZ at 267.96 nm (AML 10 µg/ml + OLM 10 µg/ml used as double divisor)

Assay of Tablet Formulation by Double Divisor Ratio Spectra Derivative Spectrophotometry

Content of Twenty Tablets were weighed accurately. A powder quantity equivalent to 16 mg OLM, 4 mg AML and 10 mg HCTZ was accurately weighed and transferred to volumetric flask of 25 ml capacity. 15 ml of Methanol was transferred to this volumetric flask and sonicated for 20min. The flask was shaken and volume was made up to the mark with diluent. The above solution was filtered through whatman filter paper (0.45µ). The solution was made up to the mark with diluent to give a solution containing 16 µg/ml of OLM, 4 µg/ml of AML and 10 µg/ml of HCTZ. The resulting solution was analyzed by proposed method. The quantitation was carried out by keeping these values to the straight line equation of calibration curve.

Method Validation

The proposed method has been extensively validated in terms of linearity, accuracy, precision, reproducibility, Limit of Detection (LOD) and Limit of Quantification (LOQ).

1. Linearity

Aliquots from OLM, AML and HCTZ standard stock solution was transferred to volumetric flask of 10ml capacity. The volume was adjusted upto the mark with diluent to give a solution containing 4-20 µg/ml OLM, 4-20 µg/ml AML and 3-15 µg/ml HCTZ. All divided and derivatized Spectrum were recorded using above spectrophotometric condition. Absorbance at 258.98 nm, 348.95 nm and 267.96 nm were recorded for OLM, AML and HCTZ respectively. The solutions were analyzed and absorbance was measured and calibration curves were plotted against drug concentration.

2. Precision

The intra-day and inter-day precisions of the proposed method was determined by analyzing corresponding responses in triplicate on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of OLM (4, 12 and 20 µg/ml), AML (4, 12 and 20 µg/ml) and HCTZ (3, 9 and 15 µg/ml). Results were reported in terms of % RSD.

3. Accuracy

The accuracy of the method was determined by calculating recovery of OLM, AML and HCTZ by the standard addition method. Known amounts of standard solutions of OLM (12.8, 16, 19.2 µg/ml), AML (3.2, 4, 4.8 µg/ml) and HCTZ (8, 10, 12 µg/ml) were added in pre-analyzed sample solution of marketed formulation (OLM 16µg/ml, AML 4µg/ml and HCTZ 10µg/ml) which gives solution having strength of 80%, 100% and 120% of middle concentration from the range. Each solution was analyzed in triplicates and recovery was calculated by measuring absorbance.

4. Limit of Detection (LOD) and limit of Quantification (LOQ)

The limit of Detection (LOD) and limit of quantitation (LOQ) of the drug were calculated using following equations as per ICH guideline.

$$\text{LOD} = \frac{3.3\ \sigma}{S} \qquad \text{LOD} = \frac{10\ \sigma}{S}$$

Where,
σ is Standard deviation of the response and S is slope of the calibration curve.

Results and Discussion

Method Development and Validation

The overlain derivative amplitude of double divisor ratio spectra of the drugs suggested that this method was a suitable for simultaneous determination of Olmesartan Medoxomil, Amlodipine Besylate and Hydrochlorthiazide. Methanol: Water (70:30) was taken as solvent for all three drugs. Optimized method parameters for ratio derivative spectrophotometry are shown in table 1.

Table 1: Optimized method parameters for ratio derivative spectrophotometry

Method parameters	Optimized parameters
Solvent	Methanol :Water(70:30)
Scanning range	200 nm to 400 nm
Scan speed	Medium
Δλ for tracing	10 nm
Divisor conc. for determination of OLM	10 µg/ml of each AML and HCTZ
Divisor conc. for determination of AML	10 µg/ml of each OLM and HCTZ
Divisor conc. for determination of HCTZ	10 µg/ml of each OLM and AML
Analytical wavelength for determination of OLM	258.98 nm
Analytical wavelength for determination of AML	348.95 nm
Analytical wavelength for determination of HCTZ	267.96 nm

Linearity

The calibration curves of OLM, AML and HCTZ were linear in the range of 4-20 µg/ml, 4-20 µg/ml and 3-15 µg/ml respectively. The regression equations and regression coefficients of calibration curves were

$y = -0.0052x - 0.0020$, $R^2 = 0.9992$ for OLM,
$y = 0.1350x - 0.0563$, $R^2 = 0.9980$ for AML,
$y = 0.0195x + 0.0424$, $R^2 = 0.9976$ for HCTZ.

Precision

Relative standard deviation (% RSD) for repeatability was found to be 1.099 %, 0.209 % and 0.991 % for OLM, AML and HCTZ respectively. The intraday precision showed % RSD in the range of 0.501 - 0.285 % for OLM, 0.565 - 0.546 % for AML and 0.566 - 0.764 % respectively. The inter day precision showed % RSD ranging from 1.145 -0.881 % for OLM, 1.030 - 1.225 % for AML and 0.798 - 1.386 % for HCTZ respectively. Results of intraday and inter day precision of method is illustrated in Table 2.

Table 2: Validation Parameters for Double Divisor Ratio derivative spectrophotometry

Parameters	OLM	AML	HCTZ
Linearity range	4-20 µg/ml	4-20 µg/ml	3-15 µg/ml
Regression equation	y = -0.0052x - 0.0020	y = 0.1350x - 0.0563	y = 0.0195x + 0.0424
Correlation co-efficient	0.9992	0.9980	0.9976
Precision (% RSD)			
Intraday (n=3)	0.501 - 0.285	0.565 - 0.546	0.566 - 0.764
Interday (n=3)	1.145 - 0.881	1.030 - 1.225	0.798 - 1.386
Accuracy or % Recovery	101.98 – 101.07 %	101.35 - 101.75 %	99.02 - 100.80 %
LOD			
By calculation	0.408 µg/ml	0.447 µg/ml	0.224 µg/ml
LOQ			
By calculation	1.236 µg/ml	1.355 µg/ml	0.388µg/ml

Accuracy

The percentage recovery of drugs from marketed formulation was determined by standard addition of pure drugs at three known concentrations and excellent recovery was obtained at each concentration level. The mean recoveries were found in range of 101.98 – 101.07%, 101.35 – 101.75 % and 99.02 – 100.80 % OLM, AML and HCTZ, respectively The results of accuracy studies are shown in table 3.

Table 3: Accuracy studies

Drug	%Level	Conc. of sample taken (µg/ml)	Conc. of pure Std. added (µg/ml)	Total Conc. (µg/ml)	Mean Total Conc. found(µg/ml)	% Recovery Mean*
OLM	80	16	12.8	28.8	28.97	101.98
	100	16	16.0	32.0	31.99	100.41
	120	16	19.2	35.2	35.33	101.07
AML	80	4	3.2	7.2	7.31	101.35
	100	4	4.0	8.0	8.12	101.33
	120	4	4.8	8.8	8.95	101.75
HCTZ	80	10	8.0	18.0	17.75	99.02
	100	10	10.0	20.0	19.78	99.54
	120	10	12.0	22.0	21.83	100.80

*Mean of three estimations

LOD and LOQ (Limit of Detection and Limit of Quntificaytion)

The LOD as calculated by standard formula was found to be 0.408µg/ml, 0.447µg/ml and 0.224µg/ml for OLM, AML and HCTZ respectively. The LOQ as calculated by standard formula was found to be 1.236µg/ml, 1.355µg/ml and 0.388µg/ml for OLM, AML and HCTZ respectively.

Application of the Method in Tablets

The proposed UV method was applied for the determination of Olmesartan Medoxomil, Amlodipine Besylate and Hydrochlorthiazide in their combined pharmaceutical formulation and the results are shown in table 4. The percentage recovery values (98.10-100.79 %) confirm the suitability of the proposed method for the routine determination of these components in combined formulation.

Table 4: Results of simultaneous estimation of OLM, AML and HCTZ in marketed formulation by Double divisor ratio derivative spectrophotometry method.

Drugs	Label claim (mg)	Amount of drug estimated (mg/tab)	%Label claim ± SD	%RSD
OLM	20	19.90	99.51 ± 0.1338	0.134
AML	5	5.08	101.75 ± 0.1214	0.119
HCTZ	12.5	12.49	99.94 ± 0.7743	0.774

SD = Standard Deviation

Conclusion

The proposed Double Divisor Ratio Derivative spectrophotometry method gives accurate and precise results for determination of OLM, AML and HCTZ in marketed formulation (tablet) without prior separation and is easily applied for routine analysis. The most striking feature of this method is its simplicity, accuracy and cost effectiveness. Method validation has been demonstrated by various tests for linearity, accuracy, precision, LOD and LOQ.

Acknowledgement

The authors are thankful to Torrent Pharmaceutical, Ahmadabad for providing pure gift samples of Olmesartan Medoxomil, Amlodipine Besylate and Hydrochlorthiazide respectively.

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