Chemometric Assisted Spectrophotometric Method for the Simultaneous Determination of Olmesartan Medoxomil and Hydrochlorothiazide in Bulk and Tablet Dosage Form

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In this work, a numerical method, based on the use of spectrophotometric data coupled to partial least squares, multivariate calibration is evaluated for the simultaneous determination of olmesartan medoxomil and hydrochlorothiazide in bulk and tablet dosage form. Tablet Olmetor-H (HTZ 12.5 mg and OLM 20.0 mg) was used in this study. The equipment used was a Ultraviolet-Visible double beam spectrophotometer with a matching pair of 1 cm quartz cell and electronic balance. Spectra of olmesartan medoxomil and hydrochlorothiazide were recorded at concentrations within their linear ranges 2.5-20 µg/ml and 4-32 µg/ml, respectively and were used to compute a total of 25 synthetic mixtures involving 16 calibration and 9 validation sets between the wavelength range of 200 nm and 350 nm with the wavelengths intervals, lambda=3 nm in methanol. The analytical performances of these chemometric methods were characterized by relative prediction errors and recovery studies (%), and were compared with each other. The proposed method is simple, rapid and can be easily used as an alternative analysis tool in the quality control of drugs and formulation. PLS was applied successfully for the simultaneous determination of OLM and HTZ in laboratory mixtures and pharmaceutical formulation.

Key words: Hydrochlorothiazide, olmesartan medoxomil, partial least squares, chemometrics, ultraviolet-visible spectrophotometry

Olmesartan Medoxomil (OLM), chemically 2,3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(O-1H-tetrazol-5-yl phenyl) benzyl] imidazole-5-carboxylate, cyclic 2,3-carbonate is a prodrug and it is hydrolysed to olmesartan during absorption from the gastrointestinal tract (fig. 1A). It is a selective Angiotensin 1 (AT1) subtype angiotensin II receptor antagonist. Hydrochlorothiazide (HTZ), chemically 6-chloro-3,4-dihydro-2,4-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide (fig. 1B), is a widely used thiazide diuretic[1-3]. Olmesartan and HTZ are available in the market as a combined dosage form for the treatment of hypertension. An extensive literature survey revealed the determination of OLM in dosage form by Ultraviolet (UV)-visible spectrophotometry[4,5], High Performance Liquid Chromatography (HPLC)-UV[6,7] and capillary electrophoresis[8] and in biological fluids by HPLC[9] and Liquid Chromatography-Mass Spectrometry (LC-MS)[10,11]. Determination

methods of HTZ in pharmaceutical dosage form and biological fluids include chemiluminescence[12], HPLC[13] and electrochemical study[14]. Determination methods of OLM and HTZ combination include UV-spectrophotometry[15-18], Reverse Phase (RP)-HPLC and High-Performance Thin Layer Chromatography (HPTLC)[19,20]. In this work, a simple, accurate, precise and inexpensive quantitative method has been developed for the simultaneous determination of the coexisting two drugs in the tablet dosage form. The method is based on a Partial Least Square (PLS) multivariate calibration chemometric procedure. Moreover, being simple and inexpensive, it is more appealing to use for

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routine assay of OLM and HTZ combination in a tablet than HPLC which is demanding in terms of running cost and sophistication.
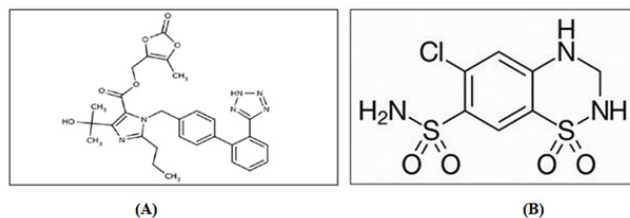

Fig. 1: Chemical structure of (A) OLM and (B) HTZ

MATERIALS AND METHODS

The reference standard of HTZ and OLM was obtained as a gratis sample from Sidmak Labs Pvt. Ltd., Valsad, Gujarat. Methanol was procured from Chemdyes Corporation, Vadodara, Gujarat. Tablet Olmetor-H (HTZ 12.5 mg and OLM 20.0 mg) was provided by Torrent Pharmaceuticals. The equipment used was a UV-Visible double beam spectrophotometer with a matching pair of 1 cm quartz cell (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) and electronic balance (Mettler Toledo). Data was acquired and processed using XLSTAT software and it was used for PLS model development and data analysis.

Preparation of standard stock solution:

Accurately weighed and transferred OLM (10 mg) and HTZ (10 mg) into two different 100 ml volumetric flask respectively and volume was made up to 100 ml with methanol up to the mark. The final concentration of OLM and HTZ were 100 µg/ml.

Preparation of working stock solution:

The standard stock solution of OLM and HTZ 100 µg/ml was used as a working solution.

Construction of calibration and validation set:

Two sets of standard solutions, a calibration set and a validation set were prepared. The multivariate calibration requires a suitable experimental design of the standard composition of the calibration set to provide the best prediction. The factorial design method was used to construct the calibration set. The application of two factorial designs led to the construction and optimization of the PLS model. Two binary sets of the drug present in the random ratio were prepared, one set with 5 samples so that a total of 25 samples were employed for optimization by PLS method by mixing appropriate volumes of the working

standard solutions of OLM and HTZ, and diluting to volume with methanol. Eight validation standard mixtures were prepared. The combination of OLM and HTZ is illustrated in Table 1. The absorption spectra of the prepared solutions were measured from 200-400 nm with 3 nm intervals. The absorbance data of the calibration set were then subjected to the XLSTAT program for the PLS model. For validation of the PLS model, the concentrations of OLM and HTZ in the validation set were predicted by using the proposed PLS model. The validation of all the methods was performed by International Council for Harmonisation (ICH) Q2 (R1) and International Union of Pure and Applied Chemistry (IUPAC) guidelines for calibration in analytical chemistry.

TABLE 1: COMPOSITION OF CALIBRATION SAMPLE OF OLM AND HTZ

Sample	Concentration (µg/ml)	
Sr. no.	OLM	HTZ
1	2.5	4
2	2.5	8
3	2.5	16
4	2.5	24
5	2.5	32
6	5	4
7	5	8
8	5	16
9	5	24
10	5	32
11	10	4
12	10	8
13	10	16
14	10	24
15	10	32
16	15	4
17	15	8
18	15	16
19	15	24
20	15	32
21	20	4
22	20	8
23	20	16
24	20	24
25	20	32

Note: OLM: Olmesartan Medoxomil and HTZ: Hydrochlorothiazide. The calibration set and validation set were randomly prepared with a mixture of OLM and HTZ in methanol

Assay of marketed formulation:

Twenty tablets were weighed and the average weight was calculated. The tablets were triturated thoroughly and mixed. Tablet powder equivalent to 12.5 mg of HTZ and 20.0 mg of OLM, based on label claim was

transferred to a 50.0 ml volumetric flask, dissolved by sonication for 15 min with enough methanol, and volume was made up to mark with methanol. The content was filtered through Whatman filter paper (No. 41). A 10.0 ml portion of the above filtrate was further diluted to 50.0 ml with distilled water. A 10.0 ml portion of this solution was further diluted to 50.0 ml with distilled water. The analysis procedure was repeated six times for tablet formulation and the results were shown below.

RESULTS AND DISCUSSION

Calibration matrix and selection of spectral zones for analysis by PLS was shown here. Fig. 2 shows the UV spectra for OLM and HTZ individual and the mixture of them in methanol. As shown, there is clear overlapping between them. The spectral overlapping of these drugs prevents the resolution of the mixtures by direct spectrophotometric measurements. OLM exhibits absorption maxima at 257 nm and HTZ exhibits absorption maxima at 225 nm. The OLM and HTZ spectra are overlapped in the absorption maxima.

The first step in multivariate methods involved constructing the calibration matrix. The wavelength range used was 200-350 nm. 52 spectral points with 3 nm intervals were selected within this range. The compositions of the calibration mixtures were randomly designed to collect maximum information from the spectra of these mixtures.

The quality of multicomponent analysis is dependent on the wavelength range and spectral mode used. The UV absorption spectra of HTZ, OLM at their nominal

concentrations are shown in fig. 2. The calibration set and validation set were randomly prepared with a mixture of OLM and HTZ in methanol (Table 1). The UV spectra were observed and the absorbances were measured at 52 wavelength points in the region between 200-350 nm with 3 nm intervals. The calibration data obtained from the experimental were gathered in a matrix data by Microsoft Office Excel (Version-1811) (all the data were transposed in Microsoft Office Excel). All these data are subjected to PLS treatment by XLSTAT (Ver. 2019). The predicted concentrations of the components in each sample were compared with the actual concentrations of the components in each of the validation samples and Standard Deviation (SD), mean and Relative Standard Deviation (RSD) was calculated (Table 2 and Table 3).

PLS regression, uses the two-block predictive PLS model to model the relationship between two matrices, X and Y. Also, Partial Least Squares Regression (PLSR) models the structure of X and of Y, which gives richer results than the traditional multiple regression approach. PLSR and similar approaches provide quantitative multivariate modeling methods, with inferential possibilities similar to multiple regression, t-tests and Analysis of Variance (ANOVA). The evaluation of the predictive abilities of the models was performed by plotting the actual known concentrations against the predicted concentrations and the plot of the actual known concentrations against the predicted concentrations are mentioned in fig. 3A-fig. 3C for OLM and fig. 4A-fig. 4C for HTZ. Standardized validation matrix and predicted validation matrix of OLM and HTZ are mentioned in fig. 5A-fig. 5D.
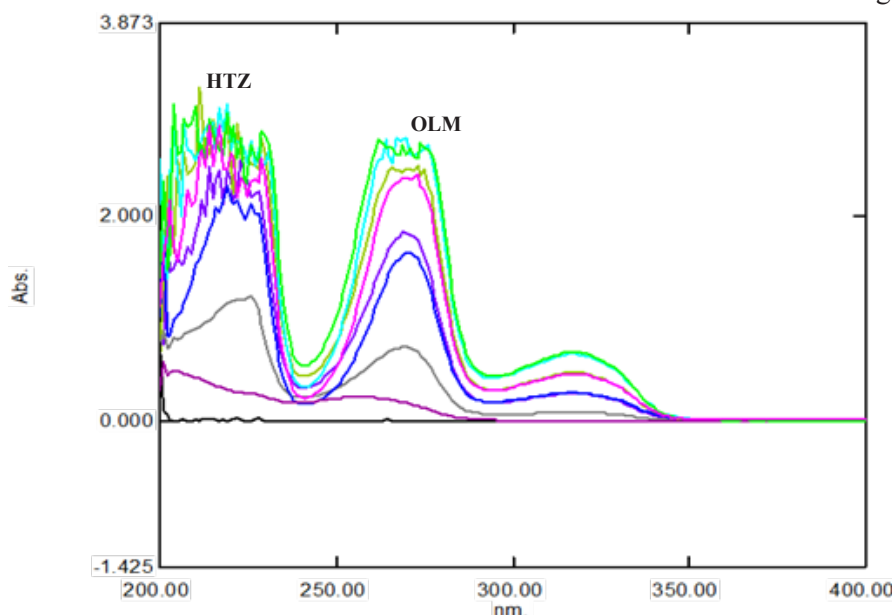

Fig. 2: Overlay spectra of OLM and HTZ. The UV spectra for OLM and HTZ individually and the mixture of them in methanol

TABLE 2: STATISTICAL ANALYSIS OF OLM BY PLS METHOD

Observation	Weight	Actual value	Predicted value	Residual matrix	Standard residual matrix	SD	Mean	RSD
Obs1	1	2.5	1.089	1.411	0.239			
Obs2	1	2.5	1.519	0.981	0.166	1.0208	2.313	50.963
Obs3	1	2.5	3.278	-0.778	-0.132			
Obs4	1	2.5	3.366	-0.866	-0.147			
Obs5	1	5	3.854	1.146	0.194			
Obs6	1	5	4.838	0.162	0.027			
Obs7	1	5	6.487	-1.487	-0.252	1.25645	5.6434	22.264
Obs8	1	5	6.85	-1.85	-0.313			
Obs9	1	5	6.188	-1.188	-0.201			
Obs10	1	10	6.188	3.812	0.645			
Obs11	1	10	9.395	0.605	0.102			
Obs12	1	10	11.383	-1.383	-0.234	2.2574	9.9088	22.781
Obs13	1	10	11.672	-1.672	-0.283			
Obs14	1	10	10.906	-0.906	-0.153			
Obs15	1	15	16.623	-1.623	-0.275			
Obs16	1	15	16.687	-1.687	-0.285			
Obs17	1	15	15.415	-0.415	-0.07	0.98631	15.697	6.283
Obs18	1	15	15.449	-0.449	-0.076			
Obs19	1	15	14.314	0.686	0.116			
Obs20	1	20	18.72	1.28	0.217			
Obs21	1	20	19.662	0.338	0.057			
Obs22	1	20	19.517	0.483	0.082	0.7998	18.8998	4.231
Obs23	1	20	18.95	1.05	0.178			
Obs24	1	20	17.65	2.35	0.398			

Note: PLSR: Partial Least Squares Regression, SD, mean and RSD are mentioned for each observation

TABLE 3: STATISTICAL ANALYSIS OF HTZ BY PLS METHOD

Observation	Weight	Actual value	Pred (4)	Residual matrix	Standard residual matrix	SD	Mean	RSD
Obs1	1	8.000	2.781	5.219	0.552			
Obs2	1	16.000	16.536	-0.536	-0.057	12.350	22.64	54.54
Obs3	1	24.000	22.765	1.235	0.131			
Obs4	1	32.000	28.626	3.374	0.357			
Obs5	1	4.000	3.520	0.480	0.051			
Obs6	1	8.000	7.638	0.362	0.038			
Obs7	1	16.000	15.568	0.432	0.046	10.342	15.610	66.25
Obs8	1	24.000	22.838	1.162	0.123			
Obs9	1	32.000	28.489	3.511	0.371			
Obs10	1	4.000	28.489	-24.489	-2.589			
Obs11	1	8.000	9.489	-1.489	-0.157			
Obs12	1	16.000	15.410	0.590	0.062	7.954	20.22	39.33
Obs13	1	24.000	20.720	3.280	0.347			
Obs14	1	32.000	27.014	4.986	0.527			
Obs15	1	4.000	6.681	-2.681	-0.283			
Obs16	1	8.000	9.779	-1.779	-0.188			
Obs17	1	16.000	16.491	-0.491	-0.052	8.389	16.40	51.15
Obs18	1	24.000	22.235	1.765	0.187			
Obs19	1	32.000	26.850	5.150	0.545			
Obs20	1	4.000	6.541	-2.541	-0.269			
Obs21	1	8.000	10.636	-2.636	-0.279			
Obs22	1	16.000	16.915	-0.915	-0.097	8.4955	16.81	50.53
Obs23	1	24.000	22.634	1.366	0.144			
Obs24	1	32.000	27.355	4.645	0.491			

Note: PLSR is Partial Least Squares Regression, SD, mean and RSD are mentioned for each observation
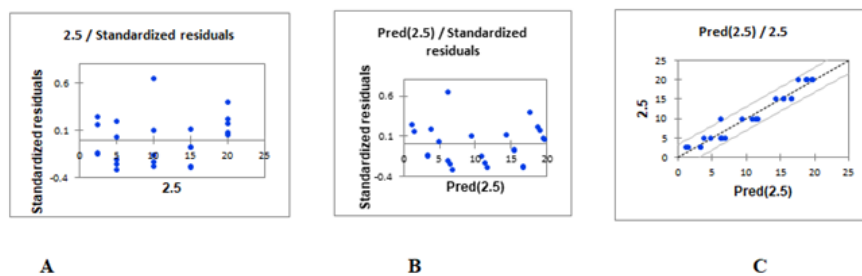
Fig. 3: (A) Standard residual matrix of OLM; (B) Predicted residual matrix of OLM and (C) Correlation matrix between the actual and predicted concentration of OLM
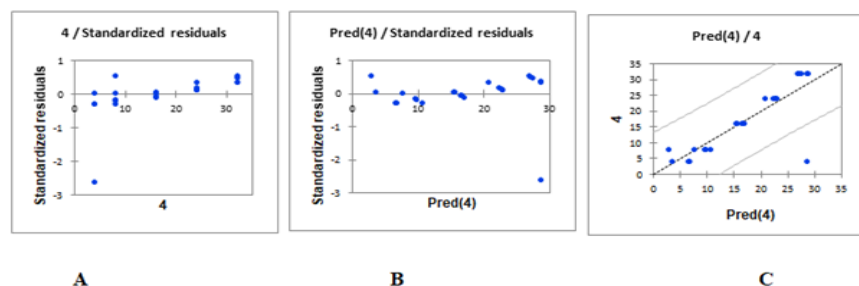

Fig. 4: (A) Standard residual matrix of HTZ; (B) Predicted residual matrix of HTZ and (C) Correlation matrix between the actual and predicted concentration of HTZ
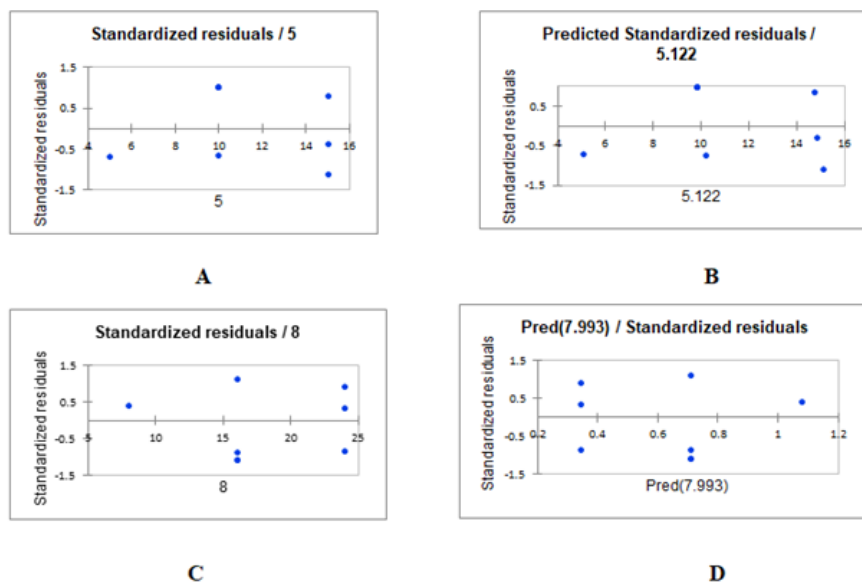

Fig. 5: (A) Standardized validation matrix of OLM; (B) Predicted validation matrix of OLM; (C) Standardized residual matrix of HTZ and (D) Predicted validation matrix of HTZ

The accuracy of the method was carried out at three levels 80 %, 100 % and 120 % of the working concentration of the sample. A calculated amount of standard solution of OLM and HTZ were spiked with added sample solution to prepare level 80 %, 100 % and 120 % of the working concentration. The analysis procedure was repeated three times. The result was shown in Table 4. The statistical parameters of the validation set and calibration set were illustrated in Table 5 and Table 6.

The validated chemometrics-assisted UV spectrophotometric methods were used in the analysis of the marketed formulation Olmetor-H with a label claim of 12.5 mg of HTZ and 20.0 mg of OLM per tablets. The results for drug assays show good agreement with the label claims (Table 7).

PLS chemometric method was applied successfully to the simultaneous determination of OLM and HTZ in the pharmaceutical dosage form. The summary parameters of the chemometric method were shown in Table 8.

TABLE 4: ACCURACY DATA OF OLM AND HTZ

Level of spiking	Blank	Amount of standard drug added		Amount of drug recovery		% Recovery	
		OLM	HTZ	OLM	HTZ	OLM	HTZ
Unspiked							
80	0	4	7.6	3.97±0.02	7.58±0.56	98.93±0.87	99.47±1.54
100	0	5	8	4.98±0.02	7.96±0.39	99.85±0.20	99.65±0.25
120	0	6	9.6	5.59±0.02	9.57±0.20	99.63±0.37	99.80±1.68

Note: Percent recovery is expressed at 3 different levels

TABLE 5: VALIDATION SET ANALYSIS BY PLS METHOD FOR OLM

Observation	Weight	Actual value	Predicted value	Residual matrix	Standard residual matrix	Mean	SD
1	1	5	5.122	-0.122	-5.7547	-0.015	0.0212
2	1	5	5.092	-0.092	-4.3396		
3	1	10	9.841	0.159	0.76849		
4	1	10	9.844	0.156	0.75399	0.038	0.2069
5	1	10	10.201	-0.201	-0.9715		
6	1	15	15.122	0.122	1.88854		
7	1	15	14.83	0.17	2.63158	0.18	0.0646
8	1	15	14.75	0.25	3.86997		

Note: Statistical parameters of the validation set and calibration set were illustrated

TABLE 6: VALIDATION SET ANALYSIS BY PLS METHOD FOR HTZ

Observation	Weight	Actual value	Predicted value	Residual matrix	Standard residual matrix	Mean	SD
1	1	8	7.993	0.007	0.12374	0.047	0.05657
2	1	8	7.913	0.087	1.53982		
3	1	16	16.024	-0.024	-0.4932		
4	1	16	16.012	-0.012	-0.2466	-0.0116	0.04866
5	1	16	15.905	0.095	1.95232		
6	1	24	23.954	0.046	1.36905		
7	1	24	23.976	0.024	0.71416	0.01666	0.03361
8	1	24	24.02	-0.02	-0.5951		

Note: PLSR is Partial Least Squares Regression, statistical parameters of the validation set and calibration set were illustrated

TABLE 7: ASSAY RESULTS OF OLM AND HTZ

Label claim		% Label claim	
HTZ	OLM	HTZ	OLM
12.5	20	101.2	99.8
		99.8	102
		100.9	100.9
		101.2	101.2
		101.6	101.6
Mean		100.98	100.66
SD±		0.61	0.82
RSD		0.6	0.81

Note: Marketed formulation Olmetor-H with a label claim of 12.5 mg of HTZ and 20.0 mg of OLM per tablets

TABLE 8: SUMMARY OF UV SPECTROPHOTOMETRIC METHOD USING PLS METHOD

Parameters	OLM	HTZ
	PLS	PLS
Range (µg/ml)	2.5-20	4-32
Wavelength (nm)	200-350	
Δλ (nm)	3	3
Factor	7	7
% recovery	98.93±99.85	99.47±99.80
Assay	100.66	100.98

Note: The summary of the UV method is expressed

Conventional multi-component UV spectroscopic methods are not suitable for combination drugs having a narrow difference in absorption maxima lambda (λ_{max}). In such cases, chemometric method serves as an alternative to other sophisticated methods like HPLC. When once the calibration matrix is built and stored in the data computation device, the samples can simply be prepared, diluted and absorbance was measured and the concentration of the sample was read from the stored matrix. PLS was applied successfully for the simultaneous determination of OLM and HTZ in laboratory mixtures and pharmaceutical formulation.

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Conflict of interests:

The authors declared no conflicts of interest.

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