IJPSR (2018), Volume 9, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 July, 2017; received in revised form, 10 September, 2017; accepted, 10 March, 2018; published 01 April, 2018

SIMULTANEOUS ESTIMATION OF TELMISARTAN, HYDROCHLORTHIAZIDE AND AMLODIPINE IN BULK AND SOLID DOSAGE FORM BY CHEMOMETRIC ASSISTED SPECTROPHOTOMETRIC METHODS

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Keywords:

Chemometrics, UV-Visible, Simultaneous, Telmisartan, Hydrochlorothiazide, Amlodipine

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ABSTRACT: Chemometric designs were applied to develop a simple UV-visible spectroscopic method for the simultaneous estimation of Hydrochlorthiazide (HCT), Amlodipine (AMLO) and Telmisartan (TEL) in bulk and solid dosage form. The simultaneous spectroscopic method was developed for the three drugs and the data generated from the spectra were determined by using Chemometric methods such as trilinear regression analysis, Cramer's matrix method, Method of least squares, Multivariate calibration methods such as partial least square regression (PLS) and Principle component regression (PCR). The wavelengths selected for all the above methods were 270 nm (wavelength of maximum absorption; λ_{max} of HCT), 342 nm (wavelength of maximum absorption; λ_{max} of AMLO) and 292 nm (wavelength of maximum absorption; λ_{max} of TEL). **Results:** The methods shows good linearity for TEL from 4 - 20 µg/ml, for HCT from 2-10 µg/ml and AMLO from 2 - 10 µg/ml with regression coefficient values of 0.970, 0.996 and 0.980 respectively. The RSD value for intraday and inter-day precision was found to be less than 2%. The percentage recovery and percentage assay was in the range of 95 - 105% for Telmisartan (TEL), Hydrochlorthiazide (HCT) and Amlodipine (AMLO) by all the methods. **Conclusion:** The developed methods neither require any oppressive separation procedure nor complex derivatization procedures for the analysis of the three drugs and moreover they are effective in minimizing the errors in analysis, simple and economical.

INTRODUCTION: Chemometrics is a branch of science which derives the data by the application of mathematical and statistical tools for the extraction of useful information from the physical and chemical phenomenon involved in a manufacturing process. Chemometrics [1, 2, 3, 4, 5] is used for calibration, signal correction and compression, pattern classification and recognition, multi variate data collection and analysis protocols, process modelling and statistical process control.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(4).1683-91

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(4).1683-91

To overcome the significant problems in the analysis of intricate multi component formulations by conventional UV-spectroscopy [6, 7, 8], HPLC [9, 10, 11, 12, 13, 14, 15, 16, 17] methods. Chemometric assisted analytical methods [18, 19, 20, 21] are designed to perform analytical investigation of such complex formulations. Telmisartan is 4' - ([4-methyl-6-(1-methyl-lH-benzimidazol-2yl)-2-propyl-lH-benzimi-dazol-l-yl] methyl}-2-biphenylcarboxylic acid. It acts as antihypertensiveand was used in treatment of hypertension.

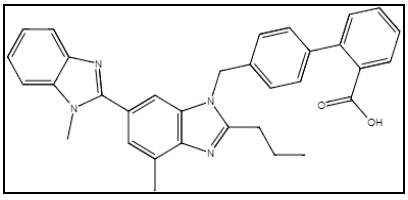

FIG. 1: STRUCTURE OF TELMISARTAN

Hydrochlorothiazide is 6-chloro-3, 4-dihydro-2H-1, 2, 4 benzothiadiazine-7-sulphonamide 1, I-dioxide. It act as diuretic.

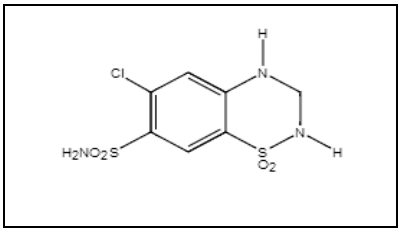

FIG. 2: STRUCTURE OF HYDROCHLORTHIAZIDE

Arnlodipine is 3-ethyE=methyl *(4RS)*-2-[(2amino-ethoxy) methyl]- 4- (2chlorophenyl)- 6- methyl-1, 4 dihydropyridine-3, 5-dicarboxylate benzene sulphonate. It comes under category of anti-hypertensive; antianginal.

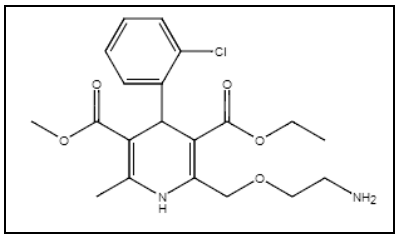

FIG. 3: STRUCTURE OF AMLODIPINE

The combination of these three drugs was widely used in the preparation of tablets to treat hypertension.

Literature survey revealed that very few analytical methods like UV-spectroscopy and HPLC methods were reported and no Chemometric methods were reported for the analysis of above combination. The present study aims to design chemometric assisted spectroscopic methods for the intricate analysis of Telmisartan, Hydrochlorthiazide, Amlodipine.

MATERIALS AND METHODS:

Instruments used: Analytical balance, UV-Visible spectrophotometer (Lab India -3072).

Data Handling Systems: UV-win for the handling of spectrophotometer, The Unscrambler X**,** Microsoft excel.

Materials Used: Working standards of drugs were procured from Dr. Reddy s laboratory.

Commercial formulation of drugs was purchased from local market. Acetonitrile AR grade was procured from Merck (India) Ltd., Mumbai.

Preparation of Solutions:

Preparation of Telmisartan Standard Solutions: 10 mg of Telmisartan standard was weighed accurately and transferred to a 25 ml volumetric flask. The sample was dissolved by using 10 ml acetonitrile by placing in sonicator for 15 min. volume was made up to the mark with acetonitrile for further dilutions were made with the telmisartan to get required concentrations of 4, 8, 12, 16 and 20 µg/ml.

Preparation of Hydrochlorthiazide Standard Solutions: 10 mg of Hydrochlorthiazide standard was weighed accurately and transferred to a 10 ml volumetric flask. The sample was dissolved by using 5 ml acetonitrile and volume was made up to the mark with acetonitrile. Further dilutions were made with the acetonitrile to get required concentrations of 2, 4, 6, 8 and 10 µg/ml.

Preparation of Amlodipine Standard Solutions: 10 mg of Amlodipine standard was weighed accurately and transferred to a 10 ml volumetric flask. The sample was dissolved by using 5 ml acetonitrile and volume was made up to the mark with acetonitrile Further dilutions were made with acetonitrile to get required concentrations of 2, 4, 6, 8 and 10 µg/ml.

Preparation of Telmisartan, Hydrochlorthiazide and Amlodipine: Stock solution was prepared by diluting 5 ml of marketed liquid formulation to 50 ml with acetonitrile. Required quantity of this stock solution was pipetted into volumetric flask to get 4 µg/ml, 2.5 µg/ml, 2 µg/ml of Telmisartan, Hydrochlorthiazide and Amlodipine respectively.

Design of Chemometric Models: Chemometric models were designed for the developed spectrophotometric methods for the simultaneous estimation of Telmisartan (TEL), Hydro-chlorthiazide (HCT) and Amlodipine (AMLO).

Trilinear Regression Analysis (TLRC): In this method three wavelengths were considered for the analysis of the component mixture [TEL(X), HCT(Y), AMLO(Z)]. The three linear regression equations were obtained by using the absorbance measured at three wavelengths against concentrations of standard solutions for each component. The slope values obtained from the

linear regression analysis for each component were used for the formation of matrix set.

The wavelengths selected for analysis were 292nm (λ_{max} of TEL), 270nm (λ_{max} of HCT), 342nm (λ_{max} of AMLO).

Equations for the formation of matrix are:

$A_{mix1} = b_{x1}C_x + b_{y1}C_y + b_{z1}C_z + a_{xyz1}$
$A_{mix2} = b_{x2}C_x + b_{y2}C_y + b_{z2}C_z + a_{xyz2}$
$A_{mix3} = b_{x3}C_x + b_{y3}C_y + b_{z3}C_z + a_{xyz3}$

Where, A_{mix1}, A_{mix2}, A_{mix3} are the absorbance of the mixture of X, Y, Z analytes at three wavelengths set. a_{xyz1}, a_{xyz2}, a_{xyz3} are the sum of intercepts of the linear regression equation at the three wavelengths.

Conversion of equation into matrix form:

$$\begin{bmatrix} Amix1- & axyz1 \\ Amix2- & axyz2 \\ Amix3- & axyz3 \end{bmatrix} = \begin{bmatrix} bx1 & by1 & bz1 \\ bx2 & by2 & bz2 \\ bx3 & by3 & bz3 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \\ Cz \end{bmatrix}$$

Cramer's Matrix Method: Molar absorptivity (ε) values were calculated by using the absorbance measured at 292 nm, 270 nm, and 342 nm for each compound in the ternary mixture. The selected wavelength values were λ_{max} of TEL, HCT and AMLO respectively. By using absorptivity (ε) values, a system of equations with three unknowns in the ternary mixture have been written as follows:

$A_{m,\,292} = \varepsilon_{TEL,\,292}\, C_{TEL} + \varepsilon_{HCT,\,292}\, C_{HCT} + \varepsilon_{AMLO,\,292}\, C_{AMLO}$
$A_{m,\,270} = \varepsilon_{TEL,\,270}\, C_{TEL} + \varepsilon_{HCT,\,270}\, C_{HCT} + \varepsilon_{AMLO,\,270}\, C_{AMLO}$
$A_{m,\,342} = \varepsilon_{TEL,\,342}\, C_{TEL} + \varepsilon_{HCT,\,342}\, C_{HCT} + \varepsilon_{AMLO,\,342}\, C_{AMLO}$

Where A_m denotes the absorbance of the ternary mixture and ε represents the values of molar absorptivity for the calculated TEL, HCT and AMLO respectively at 292nm, 270nm and 342nm. C is the molar concentration of TEL, HCT and AMLO.

The matrix simplifies and solves the system of equations with three unknowns as follows:

$$\begin{bmatrix} Am,292 \\ Am,270 \\ Am,342 \end{bmatrix} = \begin{bmatrix} \varepsilon\, TEL,292 & \varepsilon\, HCT,292 & \varepsilon\, AMLO,292 \\ \varepsilon\, TEL,270 & \varepsilon\, HCT,270 & \varepsilon\, AMLO,270 \\ \varepsilon\, TEL,342 & \varepsilon\, HCT,342 & \varepsilon\, AMLO,\ 342 \end{bmatrix} \times \begin{bmatrix} C\ TEL \\ C\ HCT \\ C\ AMLO \end{bmatrix}$$

This matrix can be solved and each compound was determined by solving the following operations

(Δ = Determinant value of matrix)

$$\Delta = \begin{bmatrix} \varepsilon\, TEL,292 & \varepsilon\, HCT,\ 292 & \varepsilon\, AMLO,292 \\ \varepsilon\, TEL,270 & \varepsilon\, HCT,\ 270 & \varepsilon\, AMLO,270 \\ \varepsilon\, TEL,342 & \varepsilon\, HCT,342 & \varepsilon\, AMLO,342 \end{bmatrix}$$

$$\Delta_1 = \begin{bmatrix} Am,292 & \varepsilon\, HCT,292 & \varepsilon\, AMLO,292 \\ Am,270 & \varepsilon\, HCT,270 & \varepsilon\, AMLO,270 \\ Am,342 & \varepsilon\, HCT,342 & \varepsilon\, AMLO,342 \end{bmatrix}$$

$$\Delta_2 = \begin{bmatrix} \varepsilon\, TEL,292 & Am,292 & \varepsilon\, AMLO,292 \\ \varepsilon\, TEL,270 & Am,270 & \varepsilon\, AMLO,270 \\ \varepsilon\, TEL,342 & Am,342 & \varepsilon\, AMLO,342 \end{bmatrix}$$

$$\Delta_3 = \begin{bmatrix} \varepsilon\, TEL,292 & \varepsilon\, HCT,292 & Am,292 \\ \varepsilon\, TEL,270 & \varepsilon\, HCT,270 & Am,270 \\ \varepsilon\, TEL,342 & \varepsilon\, HCT,342 & Am,342 \end{bmatrix}$$

By applying Cramer's matrix rule the concentration TEL, HCT and AMLO can be found by

$$C_{AMB} = \Delta_1 / \Delta,\ C_{CPM} = \Delta_2 / \Delta,\ C_{GPN} = \Delta_3 / \Delta$$

Method of Least Squares: The standard stock solutions of TEL (4 µg/ml), HCT (2.5 µg/ml) and AMLO (2 µg/ml) were measured at 265 nm, 270 nm, 275 nm, 280 nm, 285 nm, 290 nm, 295 nm, 300 nm, 305 nm, 310 nm, 315 nm, 320 nm, 325 nm, 330 nm, 335 nm, 340 nm, 345 nm, and 350 nm their absorbances were recorded (acts as calibration set) and tabulated in MS- Excel. The individual drug absorbances of known concentrations of TEL, HCT and AMLO were added and synthetic mixture (as validation set) was created and absorbances were recorded. Similarly the test sample was also measured at same wavelengths and absorbances were recorded and tabulated. By applying method of least squares using Solver add-in in MS-Excel, the actual concentration of TEL, HCT and AMLO were predicted in test samples.

Multivariate Calibration Methods: Calibration was performed by using the wavelength range 265 - 350 nm at 5 nm interval. Cross-validation of the final models was performed with respect to the number of factors affecting the prediction of each of the compounds. The optimum number of factors was found to be three for TEL, HCT and AMLO both in the both PCR and PLS models.

Validation of Spectrophotometric Method:

Linearity and Range: The linearity of analytical method is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

The range of analytical procedure is the interval between the upper and lower concentrations of the sample for which the analytical procedure has a suitable level of Precision, Accuracy and Linearity.

Precision: The precision of analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Accuracy: The accuracy of analytical procedure express the closeness or agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was determined by adding known quantities of analyte (pure drug) to the drug product and applying the developed methods to determine the quantity of the drug present in the spiked sample.

Samples were spiked with 50, 100, 150% level solutions of the standards and analysed. The experiment was performed triplicate (n = 3). Percent recovery values were reported.

$$\text{Accuracy} = \frac{\text{Amount of sample conc. found} - \text{Amount of test conc. taken}}{\text{Amount of standard conc. added}} \times 100$$

Assay: The commercial marketed formulation containing 4 mg of Telmisartan, 2.5 mg Hydro-chlorthiazide and 2 mg Amlodipine. The sample solution was treated same as standard solution. The resulting solution scanned under UV using acetenotrile as blank.

$$\text{Percent Assay} = \frac{\text{Calculated quantity of test sample (mg)}}{\text{Weight of test sample (mg)}} \times 100$$

RESULTS AND DISCUSSION:

Trilinear Regression Analysis:

TABLE 1: ABSORBANCE OF TELMISARTAN AT 292 nm, 270 nm AND 342 nm

Conc. (µg/ml)	292 nm	270 nm	342 nm
4	0.198	0.124	0.016
8	0.353	0.209	0.018
12	0.576	0.362	0.052
16	0.730	0.434	0.035
20	1.145	0.663	0.030
Linear Equation	y = 0.053x-0.038	y = 0.062x-0.015	y = 0.003x+0.008
R^2	0.970	0.977	0.575

TABLE 2: ABSORBANCE OF HYDROCHLORTHIAZIDE AT 292nm, 270 nm AND 342 nm

Conc. (µg/ml)	292 nm	270 nm	342 nm
2	0.043	0.198	0.023
4	0.076	0.391	0.037
6	0.077	0.544	0.025
8	0.106	0.698	0.036
10	0.131	0.936	0.040
Linear Equation	y = 0.006x+0.011	y = 0.090x+0.008	y = 0.003x+0.010
R^2	0.953	0.996	0.669

TABLE 3: ABSORBANCE OF AMLODIPINE AT 292 nm, 270 nm AND 342 nm

Conc. (µg/ml)	292 nm	270 nm	342 nm
2	0.078	0.101	0.099
4	0.182	0.223	0.211
6	0.203	0.260	0.284
8	0.294	0.316	0.373
10	0.264	0.356	0.408
Linear Equation	y = 0.014x+0.028	y = 0.035x+0.033	y = 0.041x+0.019
R^2	0.899	0.949	0.980

$$\begin{bmatrix} Amix1 - & axyz1 \\ Amix2 - & axyz2 \\ Amix3 - & axyz3 \end{bmatrix} = \begin{bmatrix} bx1 & by1 & bz1 \\ bx2 & by2 & bz2 \\ bx3 & by3 & bz3 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \\ Cz \end{bmatrix}$$

$$\begin{bmatrix} 0.33 - & (-0.001) \\ 0.473 - & (-0.026) \\ 0.144 - & (-0.037) \end{bmatrix} = \begin{bmatrix} 0.053 & 0.006 & 0.014 \\ 0.062 & 0.090 & 0.035 \\ 0.003 & 0.003 & 0.041 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \\ Cz \end{bmatrix}$$

$$\begin{bmatrix} 0.254 \\ 0.539 \\ 0.107 \end{bmatrix} = \begin{bmatrix} 0.053 & 0.006 & 0.014 \\ 0.062 & 0.090 & 0.035 \\ 0.003 & 0.003 & 0.041 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \\ Cz \end{bmatrix}$$

$$\begin{bmatrix} Cx \\ Cy \\ Cz \end{bmatrix} = \begin{bmatrix} 3.951 \\ 2.434 \\ 2.143 \end{bmatrix}$$

The concentration of Telmisartan (C_x), Hydrchlor-thiazide (C_y) and Amlodipine (C_z) present in the given formulation sample were found to be 3.951 µg/ml, 2.434 µg/ml and 2.143 µg/ml respectively.

Cramer's Matrix Method:

$$A_{mix1} = b_{x1}C_x + b_{y1}C_y + b_{z1}C_z + a_{xyz1}$$
$$A_{mix2} = b_{x2}C_x + b_{y2}C_y + b_{z2}C_z + a_{xyz2}$$
$$A_{mix3} = b_{x3}C_x + b_{y3}C_y + b_{z3}C_z + a_{xyz3}$$

$$\begin{bmatrix} Am,292 \\ Am,270 \\ Am,342 \end{bmatrix} = \begin{bmatrix} \varepsilon\, TEL,292 & \varepsilon\, HCT,292 & \varepsilon\, AMLO,292 \\ \varepsilon\, TEL,270 & \varepsilon\, HCT,270 & \varepsilon\, AMLO,270 \\ \varepsilon\, TEL,342 & \varepsilon\, HCT,342 & \varepsilon\, AMLO,342 \end{bmatrix} \times \begin{bmatrix} C\ TEL \\ C\ HCT \\ C\ AMLO \end{bmatrix}$$

By substituting the values in matrix and it was solved and each compound was determined by solving the following operations (Δ = Determinant value of matrix).

$$\Delta = \begin{bmatrix} 49500 & 21520 & 39000 \\ 31000 & 99200 & 50500 \\ 4000 & 11520 & 49500 \end{bmatrix}$$

$$\Delta_1 = \begin{bmatrix} 0.254 & 21520 & 39000 \\ 0.539 & 99200 & 50500 \\ 0.107 & 11520 & 49500 \end{bmatrix}$$

$$\Delta_2 = \begin{bmatrix} 49500 & 0.254 & 39000 \\ 31000 & 0.539 & 50500 \\ 4000 & 0.107 & 49500 \end{bmatrix}$$

$$\Delta_3 = \begin{bmatrix} 49500 & 21520 & 0.254 \\ 31000 & 99200 & 0.539 \\ 4000 & 11520 & 0.107 \end{bmatrix}$$

By applying Cramer's matrix rule the concentration of ATR, EZT and FNF were found as follows:

$$C_{TEL} = \Delta_1 / \Delta = 4.00\mu g/mL$$
$$C_{HCT} = \Delta_2 / \Delta = 2.49\mu g/mL$$
$$C_{AMLO} = \Delta_3 / \Delta = 2.00\mu g/mL$$

The concentration of Telmisartan(C_x), Hydrochlorthiazide (C_y) and Amlodipine (C_z) present in the given formulation sample were found to be 4.00 µg/ml, 2.49 µg/ml and 2.00 µg/ml respectively.

Method of Least Squares: The standard stock solutions of TEL (4 µg/ml), HCT (2.5 µg/ml), AMLO (2 µg/ml) were measured at 265 - 350 nm with 5 nm interval. Molar absorptivity's are calculated and tabulated. Further calculations are done as shown below:

	ABSORBANCES				ABSORBTIVITIES					
WAVELENGTH	TEL	HCT	AMLO	Am	TEL	HCT	AMLO	Acalc	Acalc-Am	(Acalc-Am)2
265	0.12	0.23	0.089	0.439	30000	92000	44500		-0.439	0.192721
270	0.124	0.248	0.088	0.46	31000	99200	44000	0.4585543	-0.001446	2.09009E-06
275	0.128	0.238	0.085	0.451	32000	95200	42500	0.4497556	-0.001244	1.54842E-06
280	0.154	0.15	0.082	0.386	38500	60000	41000	0.3860681	6.811E-05	4.6385E-09
285	0.178	0.098	0.072	0.348	44500	39200	36000	0.3490954	0.0010954	1.19987E-06
290	0.198	0.054	0.078	0.33	49500	21600	39000	0.3317704	0.0017704	3.13434E-06
295	0.195	0.042	0.081	0.318	48750	16800	40500	0.3197955	0.0017955	3.22397E-06
295	0.178	0.041	0.085	0.304	44500	16400	42500	0.305469	0.001469	2.15797E-06
300	0.165	0.034	0.086	0.285	41250	13600	43000	0.2863057	0.0013057	1.70479E-06
305	0.154	0.032	0.087	0.273	38500	12800	43500	0.2741281	0.0011281	1.2725E-06
310	0.114	0.031	0.085	0.23	28500	12400	42500	0.230496	0.000496	2.46039E-07
315	0.078	0.032	0.088	0.198	19500	12800	44000	0.1978463	-0.000154	2.36177E-08
320	0.056	0.03	0.086	0.172	14000	12000	43000	0.1715243	-0.000476	2.26292E-07
325	0.032	0.029	0.092	0.153	8000	11600	46000	0.1520548	-0.000945	8.93482E-07
330	0.022	0.027	0.095	0.144	5500	10800	47500	0.1428677	-0.001132	1.28215E-06
335	0.016	0.029	0.099	0.144	4000	11600	49500	0.1426962	-0.001304	1.70002E-06
340	0.008	0.025	0.087	0.12	2000	10000	43500	0.1187576	-0.001242	1.54344E-06
345	0.005	0.008	0.065	0.078	1250	3200	32500	0.0771573	-0.000843	7.10093E-07
										0.192743962
				Actual Concentartion						
	TEL =	4		TEL =	4.06677E-06					
	HCT =	2.5		HCT =	2.47616E-06					
	AMLO =	2		AMLO =	1.97385E-06					

J4 = G4*G24+H4*G25+I4*G26

FIG. 4: SCREEN SHOT OF ARRANGING DATA INTO EXCEL SHEET

Target Cell (Min)

Cell	Name	Original Value	Final Value
L22		1.54307E+12	2.47948E-05

Adjustable Cells

Cell	Name	Original Value	Final Value
G24	ABSORBTIVITIES	4	4.06677E-06
G25	ABSORBTIVITIES	2.5	2.47616E-06
G26	ABSORBTIVITIES	2	1.97385E-06

FIG. 5: SCREEN SHOT OF SOLVER REPORT

The concentration of Telmisartan(C_x), Hydrochlorthiazide (C_y) and Amlodipine (C_z) present in the given formulation sample were found to be 4.06 µg/ml, 2.47 µg/ml and 1.97 µg/ml respectively.

TABLE 4: PERCENTAGE ASSAY FOR THE THREE METHODS

		TLR		CRM		MLS	
	Actual conc. (µg/mL)	Predicted conc. (µg/mL)	Assay %	Predicted con. (µg/mL)	Assay %	Predicted conc. (µg/mL)	Assay %
TEL	4	4.03	100.75	4.02	100.05	4.05	101.25
HCT	2.5	2.60	104.00	2.48	99.2	2.47	98.8
AMLO	2	2.06	103.00	1.96	98.00	2.02	101.00

Multi Variate Calibration Techniques: Experimental design for the calibration set.

TABLE 5: CALIBRATION SET CONTAINING 15 SYNTHETIC MIXTURES OF TEL, HCT AND AMLO

Mix. no.	TEL	HCT	AMLO
Mix 1	12	6	6
Mix 2	12	2	2
Mix 3	4	2	10
Mix 4	4	10	4
Mix 5	20	6	10
Mix 6	8	4	6
Mix 7	20	4	4
Mix 8	12	8	4
Mix 9	8	10	8
Mix 10	8	8	10
Mix 11	16	10	8
Mix 12	20	8	6
Mix 13	16	6	10
Mix 14	12	10	10
Mix 15	20	10	2

Experimental Design for the Validation Set:

TABLE 6: VALIDATION SET CONTAINING 10 SYNTHETIC MIXTURES OF TEL, HCT AND AMLO

Mix. no.	TEL	HCT	AMLO
Mix 16	20	2	8
Mix 17	4	8	2
Mix 18	16	2	6
Mix 19	4	6	8
Mix 20	12	8	8
Mix 21	16	8	4
Mix 22	16	4	2
Mix 23	8	2	4
Mix 24	4	4	6
Mix 25	6	6	2

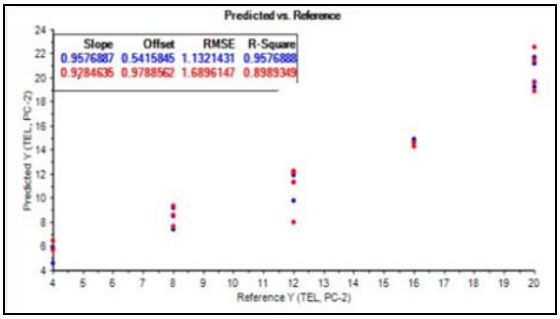

FIG. 6: PREDICTED *vs* REFERENCE CONC. OF TEL BY PCR METHOD

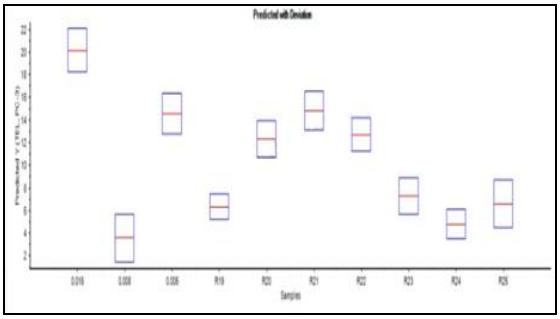

FIG. 7: PREDICTED *vs* REFERENCE CONC. OF TEL BY PCR METHOD SHOWING DEVIATION FROM MEAN

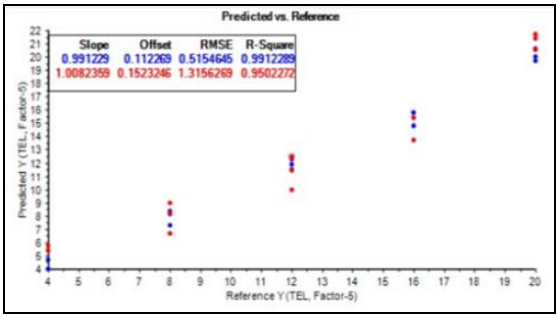

FIG. 8: PREDICTED *vs* REFERENCE CONC. OF TEL BY PLS METHOD

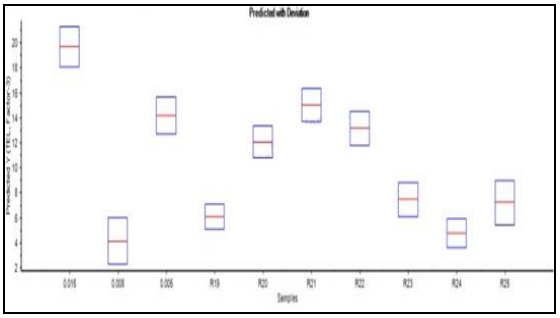

FIG. 9: PREDICTED *vs* REFERENCE CONC. OF TEL BY PLS METHOD SHOWING DEVIATION FROM MEAN

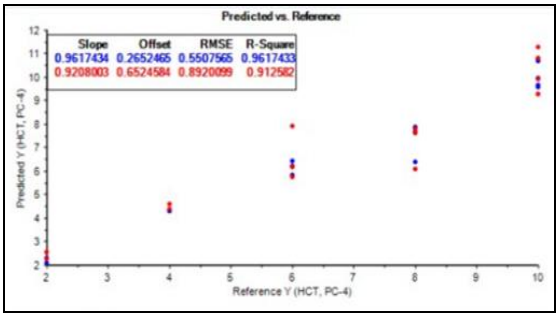

FIG. 10: PREDICTED *vs* REFERENCE CONC. OF HCT BY PCR METHOD

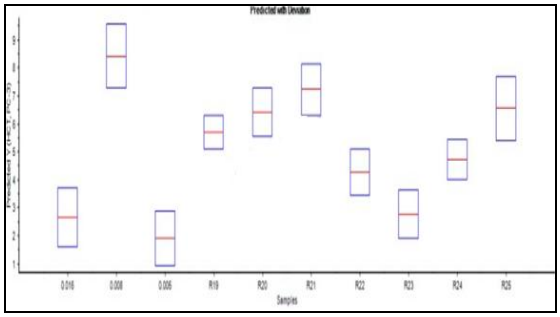

FIG. 11: PREDICTED vs REFERENCE CONC. OF HCT BY PCR METHOD SHOWING DEVIATION FROM MEAN

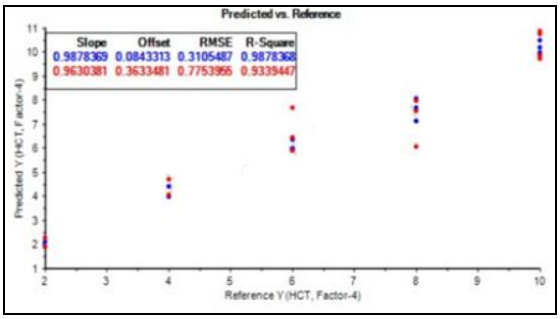

FIG. 12: PREDICTED *vs* REFERENCE CONC. OF HCT BY PLS METHOD

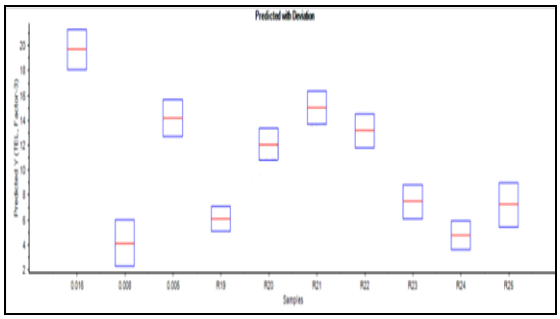

FIG. 13: PREDICTED *vs* REFERENCE CONC. OF HCT BY PLS METHOD SHOWING DEVIATION FROM MEAN

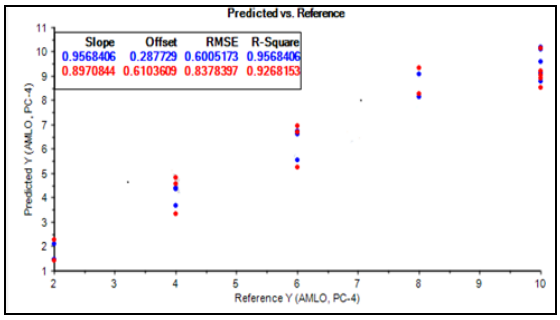

FIG. 14: PREDICTED *vs* REFERENCE CONC. OF AMLO BY PCR METHOD

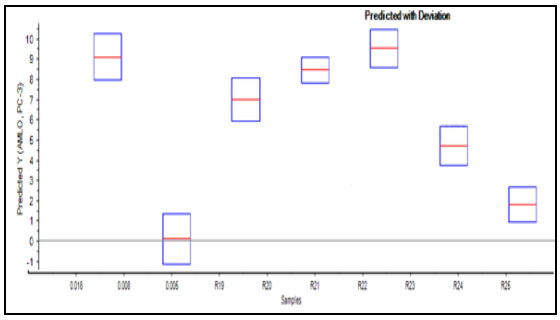

FIG. 15: PREDICTED VS REFERENCE CONC. OF AMLO BY PCR METHOD SHOWING DEVIATION FROM MEAN

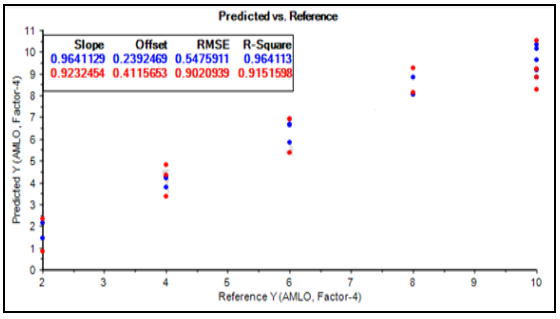

FIG. 16: PREDICTED *vs* REFERENCE CONC. OF AMLO BY PLS METHOD

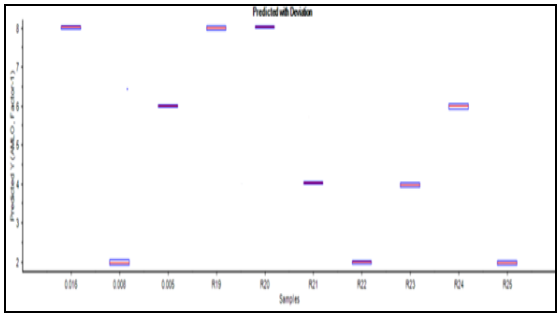

FIG. 17: PREDICTED *vs* REFERENCE CONC. OF AMLO BY PLS METHOD SHOWING DEVIATION FROM MEAN

When the calibration models were applied to the prediction set, the concentrations predicted by the models were found to be very close to the nominal concentrations, confirming the validity of both methods. The obtained results were summarized as shown below.

Assay of Pharmaceutical Formulation: From the precise prediction ability of both PCR and PLS methods the concentrations of AMB, CPM and GPN were found as follows:

TABLE 7: PREDICTED CONCENTRATIONS FROM PCR AND PLS MODELS FOR VALIDATION SET

Mix. no.	Actual Concentration (in µg/mL)			Predicted Concentration (in µg/mL)					
				PCR			PLS		
	TEL	HCT	AMLO	TEL	HCT	AMLO	TEL	HCT	AMLO
16	20	2	8	20.1057	2.6347	9.0845	19.6625	2.7352	8.0018
17	4	8	2	3.5186	8.4064	0.0845	4.1067	8.1246	1.9755
18	16	2	6	14.5405	1.8815	6.9821	14.1800	2.0557	5.9874
19	4	6	8	6.2854	5.6755	8.4462	6.0179	5.7203	7.9892
20	12	8	8	12.2360	6.3953	9.5132	12.0297	6.6592	8.0091
21	16	8	4	14.8009	7.2102	4.6845	14.9903	7.4074	4.0072
22	16	4	2	12.6487	4.2554	1.7639	13.1404	4.2397	1.9836
23	8	2	4	7.2320	2.7524	3.0959	7.4300	2.5860	3.9654
24	4	4	6	4.7565	4.7088	5.8797	4.7208	4.6524	5.9775
25	8	6	2	6.5519	6.5432	0.05382	7.1745	6.2092	1.9709

TABLE 8: PREDICTED CONCENTRATIONS FROM PCR AND PLS IN ASSAY OF FORMULATION

	PCR			PLS		
	Actual conc. (µg/mL)	Predicted conc. (µg/mL)	Assay %	Actual conc. (µg/mL)	Predicted conc. (µg/mL)	Assay %
TEL	4	4.18	104.50	4	4.10	102.5
HCT	2.5	2.48	99.20	2.5	2.49	99.60
AMLO	2	1.95	97.50	2	1.98	99.00

Acceptance criteria: 95- 105% (w/v)

Method Validation: Accuracy:

TABLE 9: PERCENTAGE RECOVERY FOR ALL THE METHODS

Drug	Percentage	% Recovery				
		For TLRC	For CRM	For MLS	For PCR	For PLS
TEL	75%	95.60	96.87	99.95	97.95	97.56
	100%	100.54	100.25	98.92	98.47	98.63
	125%	99.96	97.54	96.95	98.56	99.62
HCT	75%	95.99	97.84	99.59	98.44	98.44
	100%	100.16	99.50	97.26	99.88	98.59
	125%	99.44	97.76	100.84	96.96	97.16
AMLO	75%	96.20	98.55	97.83	98.92	99.12
	100%	99.98	98.30	100.46	99.42	98.76
	125%	99.96	100.59	99.52	96.73	97.16

Linearity and Range:

TABLE 10: LINEAR EQUATION PARAMETERS

Drug	Wave length	For TLRC Method			For Cramers matrix method(CRM)		
	nm	Linear equation	R^2	Range µg/mL	Linear equation	R^2	Range µg/mL
TEL	292	*y=0.053x-0.038*	0.970		*y=0.053x-0.038*	0.970	
	270	*y=0.062x-0.015*	0.977	4-20	*y=0.062x-0.015*	0.977	4-20
	342	*y=0.003x+0.008*	0.575		*y=0.003x+0.008*	0.575	
HCT	292	*y=0.006x+0.011*	0.953		*y=0.006x+0.011*	0.953	
	270	*y=0.090x+0.008*	0.996	2-10	*y=0.090x+0.008*	0.996	2-10
	342	*y=0.003x+0.010*	0.669		*y=0.003x+0.010*	0.669	
AMLO	292	*y=0.014x+0.028*	0.899		*y=0.014x+0.028*	0.899	
	270	*y=0.035x+0.033*	0.949	2-10	*y=0.035x+0.033*	0.949	2-10
	342	*y=0.041+0.019*	0.980		*y=0.041+0.019*	0.980	

The proposed spectrophotometric method was found to be linear and the data is presented in the **Table 10**. The intra-day and inter-day precision values for both the chemometric designs were presented in **Table 11**. Accuracy was performed in terms of the Percent recovery values and the values for Telmisartan, hydrochlorthiazide and amlodipine by all the chemometric designs were presented in **Table 9**. The assay of the commercial formulation of the drugs were performed and their percentage assay values were presented in **Table 4** and **8**.

Precision:

TABLE 11: PERCENTAGE RSD FOR ALL THE METHODS

Drug		Inter day precision (% RSD)					Intraday precision (% RSD)					
	Conc.	TLRC	CRM	MLS	PCR	PLS	TLRC	CRM	MLS	PCR	PLS	PCR
TEL	12	1.4	1.7	1.3	1.5	1.2	1.4	1.3	1.2	1.8	1.4	1.2
	16	1.4	1.5	1.4	1.4	1.4	1.3	1.5	1.3	1.6	1.6	0.9
	20	1.6	1.4	1.6	1.2	1.5	1.7	1.1	1.5	1.5	1.5	1.1
HCT	04	1.6	1.8	1.1	1.2	1.4	1.7	1.5	1.6	1.8	1.6	1.5
	06	1.5	1.6	1.2	1.3	1.6	1.6	1.7	1.5	1.7	1.7	1.6
	08	1.7	1.2	1.2	1.5	1.4	1.8	1.6	1.6	1.8	1.6	1.5
AMLO	02	1.5	1.6	1.7	1.4	1.6	1.6	1.6	1.5	1.8	1.5	1.2
	04	1.2	1.3	1.4	1.3	1.4	1.7	1.6	1.7	1.7	1.8	1.4
	06	1.5	1.2	1.3	1.4	1.2	1.6	1.8	1.5	1.6	1.6	1.6

CONCLUSION: The developed methods neither require any oppressive separation procedure nor complex derivatization procedures for the analysis of the three drugs and moreover they are effective in minimizing the errors in analysis, simple and economical. Finally it is concluded that the developed methods were simple and accurate can be used in routine analysis.

ACKNOWLEDGEMENT: We acknowledge the management and the principal of Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur for providing the facilities to carry out this research work. We also thank Dr. Reddy's laboratories, Pvt. Ltd., Hyderabad for providing the gift samples of the drugs.

CONFLICT OF INTEREST: This is a non-funding research work. There were no conflicts of interest.

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How to cite this article:
Yarramsetti S, Prabahar AE, Geervani PS and Nadendla RR: Simultaneous estimation of telmisartan, hydrochlorthiazide and amlodipine in bulk and solid dosage form by chemometric assisted spectrophotometric methods. Int J Pharm Sci & Res 2018; 9(4): 1683-91. doi: 10.13040/ IJPSR.0975-8232.9(4).1683-91.

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