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Simultaneous Estimation of Hydrochlorothiazide and Losartan Potassium in Bulk Solid Dosage Form by Chemometric Assisted Spectrophotometric Methods

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Abstract: *A simple UV-visible spectroscopic method was developed and Chemo metric designs were applied for the simultaneous estimation of hydrochlorothiazide (HCT) and losartan potassium (LST) in bulk and solid dosage form. The spectroscopic method was developed by using alcohol as solvent for the two drugs and the data generated from the spectra were mined by using Chemo metric methods such as bi-linear regression analysis, Cramer's matrix method, Method of least squares, The wavelengths selected for all the above methods were 271 nm (wavelength of maximum absorption; λmax of HCT), 213 nm (wavelength of maximum absorption; λmax of LST).*

Results: *The methods hold good linearity for HCT from 1-5μg/ml, for LST from 2-10μg/ml with regression coefficient values of 0.945 and 0.964 respectively. The intraday and inter-day precision was found to be less than 2% RSD. The percentage recovery and percentage assay was in the range 85-105% of for hydrochlorothiazide (HCT) and losartan potassium (LST) by all the methods.*

Conclusion: *The developed methods neither require any cumbersome separation procedure nor complex derivatization procedures for the analysis of the two drugs and more over they are effective in minimizing the errors in analysis, simple and economical.*

Keywords: *uv-visible spectroscopy, chemometrics, hydrochlorothiazide and losartan potassium*

I. Introduction

Chemo metrics is a branch of science that is used for extraction of the data related to chemical and physical phenomena involved in the manufacturing process by the application of the statistical and Mathematical methods.[1][4] It can be applied in predictive issues solving like predicting the target properties, desired features.[5] Also can be used for the descriptive issue solving like the model composition, identification and understanding. Chemometrics shows its application in the multivariate data collection and analysis.[6][7] Various algorithms and analogous ways are available for processing and evaluating the data.[9] They can be implemented to various fields, like medicine, pharmacy, food control, and environmental monitoring. [10][11] The primary focus of chemometrics involves the use of mathematical or software procedures in particular, both to develop analytical methods and to analyse the signals and results obtained. [12][13]

Hydrochlorothiazide:
IUPAC Name: 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide

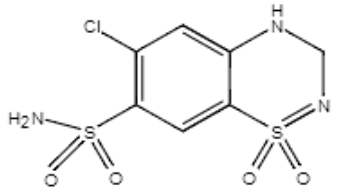


Fig No.1: Structure of hydrochlorothiazide

Losartan potassium:
IUPAC Name : potassium;[2-butyl-5-chloro-3-[[4-[2-(1,2,3-triaza-4-azanidacyclopenta-2,5-dien-5-yl)phenyl]phenyl]methyl]imidazol-4-yl]methanol

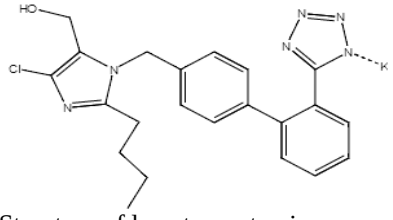


Fig No.2: Structure of losartan potassium

II. Materials And Methods:

Instruments used:

- Analytical balance
- UV-Visible spectrophotometer (Lab India -3072)

Data handling systems:

- UV-win for the handling of spectrophotometer.
- Microsoft excel.
- Easy Matrix Calculator Pro (Ver. 5.4) was used for resolving the data matrices.
- Chem diagram

Materials used:

These Working standard drugs are procured from Dr. Reddys laboratory. Commercial formulation of drugs was purchased from local market. Alcohol was procured from Merck (India) ltd, Mumbai.

PREPARATION STANDARED SOLUTIONS

Preparation of Hydrochlorothiazide standard solution:

Take 10 ml volumetric flask in 10 mg of Hydrochlorothiazide (API) and dissolve alcohol and mix it well. Then the concentration is (1000µg/ml) then take 10ml volumetric flask in 1ml of the above stock solution then con was (100µg/ml). then take10ml of volume metric flask into 100µg/ml like 0.1,0.2,0.3,0.4,0.5ml then the concentrations like 1,2,3,4,5. Prepare in 10ml of volume metric flask make up with alcohol. Observe in uv-visible spectroscopy at 271nm.

Preparation of Losartan potassium standard solution

Take 10 ml volumetric flask in 10 mg of Losartanpotassium (API) and dissolve alcohol and mix it well. Then the concentration is (1000µg/ml) then take 10ml volumetric flask in 1ml of the above stock solution then con was (100µg/ml). then take10ml of volume metric flask into 100µg/ml like 0.2,0.4,0.6,0.8,1µg/ml. then the concentrations like 2,4,6,8,10.Prepare in 10ml of volume metric flask make up with methanol. Observe in uv-visible spectroscopy at 213nm.

Preparation of Hydrochlorothiazide and Losartan potassium :

Stock solution was prepared by diluting 176mg of marketed formulation to 50ml with alcohol(10 ml volumetric flask/1000µg/ml) take 0.1ml into 10ml volumetric flask then make up into 10 ml with alcohol. From above solution take 0.2 ml then make up with methanol up to 10 ml (2 conc) was observed in uv-visible spectroscopy at 200-400nm.

DESIGN OF CHEMOMETRIC MODELS:

Chemo metric models were designed for the developed spectrophotometric methods for the simultaneous estimation of hydrochlorothiazide (HCT) andlosartan potassium (LST).

Linear Regression Component (LRC) method:

In this method two wavelengths were considered for the analysis of the component mixtures are like [HCT and LST].The two linear regression equations were obtained by using the absorbance measured at two 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 271 ($\lambda_{max}$of HCT), 213 nm (λ_{max} of LST).

Equations for the formation of matrix are:

$\mathbf{A_{mix1}=b_{x1}C_x+b_{y1}C_y+a_{xy1}}$

$\mathbf{A_{mix2}=b_{x2}C_x+b_{y2}C_y+a_{xy2}}$

Where, $\mathbf{A_{mix1}}$, $\mathbf{A_{mix2}}$ are the absorbance of the mixture of X, Y analytics at three wavelengths set. $\mathbf{a_{xy1}}$, $\mathbf{a_{xy2}}$ **are** the sum of intercepts of the linear regression equation at the three wavelengths.

Conversion of equation into matrix form:

$$\begin{bmatrix} \text{Amix1} - & \text{axy1} \\ \text{Amix2} - & axy2 \end{bmatrix} = \begin{bmatrix} \text{bx1} & \text{by1} \\ \text{bx2} & \text{by2} \end{bmatrix} \times \begin{bmatrix} \text{Cx} \\ \text{Cy} \end{bmatrix}$$

Cramer's Matrix Method

Molar absorptivity (ε) values were calculated by using the absorbance measured at 271nm, 213 nm, for each compound in the binary mixture. The selected wavelength values were λ_{max} of HCT, and LST respectively. By

using absorptivity (ε) values, a system of equations with two unknowns is the binary mixture has been written as follows:

$A_{m,\ 271} = \varepsilon_{HCT,\ 271}\ C_{HCT} + \varepsilon_{LST,\ 271}\ C_{LST}$

$A_{m,\ 213} = \varepsilon_{HCT,\ 273}\ C_{HTC} + \varepsilon_{LST,\ 273}\ C_{LST}$

Where A_m denotes the absorbance of the Two mixture and ε represents the values of molar absorptivity for the calculated, HCT and LST respectively at 271nm, 213 nm. is the molar concentration of HCT and LST.

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

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

(Δ = Determinant value of matrix)

$$\Delta = \begin{bmatrix} \varepsilon\ HCT\ 271 & \varepsilon\ LST\ 271 \\ \varepsilon\ HCT\ 213 & \varepsilon\ LST\ 213 \end{bmatrix}$$

$$\Delta_1 = \begin{bmatrix} Am271 & \varepsilon\ LSR\ 271 \\ Am213 & \varepsilon\ LST\ 213 \end{bmatrix}$$

$$\Delta_2 = \begin{bmatrix} \varepsilon\ HCT\ 271 & Am271 \\ \varepsilon\ HCT\ 213 & Am213 \end{bmatrix}$$

By applying Cramer's matrix rule the concentration HCT and LST can be found by

$$C_{HCT} = \Delta_1 / \Delta \qquad C_{LST} = \Delta_2 / \Delta$$

Method of Least Squares

The standard stock solutions of HCT (2µg/ml) and LST (8µg/ml) were measured at 210nm, 215nm,220nm,225nm,230nm,235nm,240nm,245nm,250nm,255nm,260nm,265nm,270nm, 275nm,280nm and their absorbance's were recorded (acts as calibration set) and tabulated in MS- Excel. The individual drug absorbance of known concentrations of HCT and LST 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 absorbance's were recorded and tabulated. By applying method of least squares using Solver add-in in MS-Excel, the actual concentration of HCT and LST were predicted in test samples.

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 75%100,125% level solutions of the standards and analysed. The experiment was performed triplicate (n=3). Percent recovery values were reported.

$$\text{Accuracy} = \frac{\text{Amount of Sample Conc. found} - \text{Amount of Test Conc. taken}}{\text{Amount of Standard Conc. added}}\ \text{X}\ 100$$

Assay:

The commercial marketed formulation containing hydrochlorothiazide of 2 mg.and losartan of 8 mg.The sample solution was treated same as standard solution. The resulting solution scanned under UV using methanol as blank.

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

III. Results And Discussion

BILINEAR REGRESSION ANALYSIS:

Table No.1: Absorbance of Hydrochlorothiazide at 271nm and 213nm

Conc(µg/ml)	271nm	213nm
1	0.020	0.048
2	0.042	0.096
3	0.138	0.135
4	0.209	0.176
5	0.232	0.212
Linear equation	Y=0.052x-0.023	Y=0.042x+0.005
R^2	0.945	0.996

Table No.2: Absorbance of Losartan at 213nm and 271nm

Conc(µg/ml)	213nm	271nm
2	0.064	0.011
4	0.141	0.022
6	0.219	0.030
8	0.393	0.036
10	0.523	0.055
Linear equation	Y=0.052x-0.039	Y=0.005x-0.0001
R^2	0.964	0.977

$$\begin{bmatrix} Amix1 - & axy1 \\ Amix2 - & axy2 \end{bmatrix} = \begin{bmatrix} bx1 & by1 \\ bx2 & by2 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

$$\begin{bmatrix} 0.121 - & (-0.0231) \\ 0.491 - & (+0.0385) \end{bmatrix} = \begin{bmatrix} 0.052 & 0.005 \\ 0.052 & 0.042 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

$$\begin{bmatrix} 0.144 \\ 0.452 \end{bmatrix} = \begin{bmatrix} 0.052 & 0.005 \\ 0.052 & 0.042 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

$$\begin{bmatrix} Cx \\ Cy \end{bmatrix} = \begin{bmatrix} 1.956 \\ 8.459 \end{bmatrix}$$

The concentration of Hydrochlorothiazide (C_x), and Losartan (C_y) present in the given formulation sample were found to be 1.956µg/ml and 8.456µg/ml.

Cramer's matrix method:

$A_{mix1} = b_{x1}C_x + b_{y1}C_y + a_{xy1}$

$A_{mix2} = b_{x2}C_x + b_{y2}C_y + a_{xy2}$

$$\begin{bmatrix} Am, 271 \\ Am, 213 \end{bmatrix} = \begin{bmatrix} \varepsilon\ HCT, 271 & \varepsilon\ LST, 271 \\ \varepsilon\ HCT, 213 & \varepsilon\ LST, 213 \end{bmatrix} \times \begin{bmatrix} C\ HCT \\ C\ LST \end{bmatrix}$$

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).

$$\Delta = \begin{bmatrix} 54000 & 4500 \\ 48000 & 49125 \end{bmatrix}$$

$$\Delta_1 = \begin{bmatrix} 0.144 & 4500 \\ 0.457 & 49125 \end{bmatrix}$$

$$\Delta_2 = \begin{bmatrix} 54000 & 0.144 \\ 48000 & 0.457 \end{bmatrix}$$

By applying Cramer's matrix rule the concentration of HCT and LST were found as follows

$C_{HCT} = \Delta_1 / \Delta$

$= 2.05$µg/ml

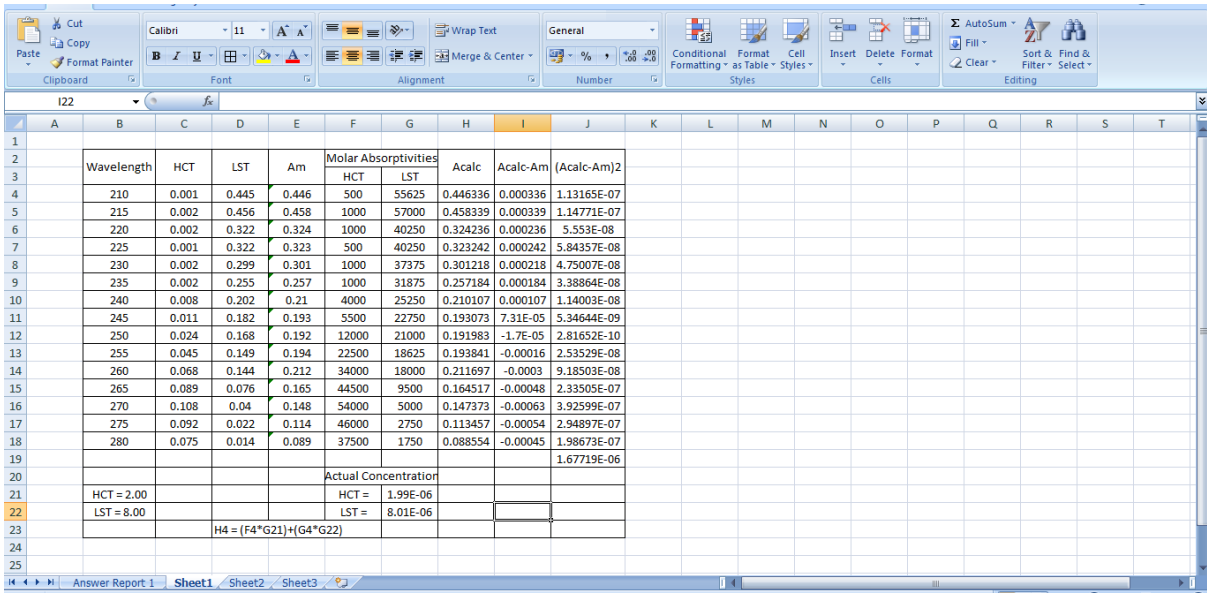
$C_{LST} = \Delta_2 / \Delta$

$= 7.290$µg/mL

The concentration of Hydrochlorothiazide (C_x), and Losartan (C_y) present in the given formulation sample were found to be 2.05µg/ml, and 7.290µg/ml respectively.

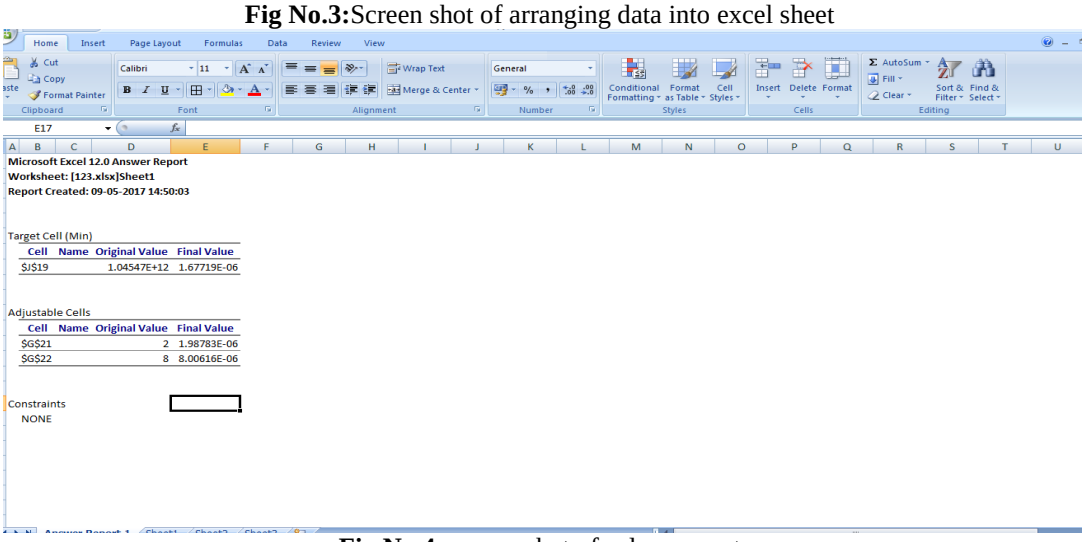
Method of least squares:

The standard stock solutions of HCT (µg/mL), LST (µg/mL), were measured at 240-280 nm with 4 nm interval. Molar absorptivity's are calculated and tabulated. Further calculations are done as shown below.



Wavelength	HCT	LST	Am	Molar Absorptivities HCT	Molar Absorptivities LST	Acalc	Acalc-Am	(Acalc-Am)2
210	0.001	0.445	0.446	500	55625	0.446336	0.000336	1.13165E-07
215	0.002	0.456	0.458	1000	57000	0.458339	0.000339	1.14771E-07
220	0.002	0.322	0.324	1000	40250	0.324236	0.000236	5.553E-08
225	0.001	0.322	0.323	500	40250	0.323242	0.000242	5.84357E-08
230	0.002	0.299	0.301	1000	37375	0.301218	0.000218	4.75007E-08
235	0.002	0.255	0.257	1000	31875	0.257184	0.000184	3.38864E-08
240	0.008	0.202	0.21	4000	25250	0.210107	0.000107	1.14003E-08
245	0.011	0.182	0.193	5500	22750	0.193073	7.31E-05	5.34644E-09
250	0.024	0.168	0.192	12000	21000	0.191983	-1.7E-05	2.81652E-10
255	0.045	0.149	0.194	22500	18625	0.193841	-0.00016	2.53529E-08
260	0.068	0.144	0.212	34000	18000	0.211697	-0.0003	9.18503E-08
265	0.089	0.076	0.165	44500	9500	0.164517	-0.00048	2.33505E-07
270	0.108	0.04	0.148	54000	5000	0.147373	-0.00063	3.92599E-07
275	0.092	0.022	0.114	46000	2750	0.113457	-0.00054	2.94897E-07
280	0.075	0.014	0.089	37500	1750	0.088554	-0.00045	1.98673E-07
								1.67719E-06
				Actual Concentration				
HCT = 2.00				HCT =	1.99E-06			
LST = 8.00				LST =	8.01E-06			
		H4 = (F4*G21)+(G4*G22)						

Fig No.3: Screen shot of arranging data into excel sheet



Microsoft Excel 12.0 Answer Report
Worksheet: [123.xlsx]Sheet1
Report Created: 09-05-2017 14:50:03

Target Cell (Min)

Cell	Name	Original Value	Final Value
J19		1.04547E+12	1.67719E-06

Adjustable Cells

Cell	Name	Original Value	Final Value
G21		2	1.98783E-06
G22		8	8.00616E-06

Constraints
NONE

Fig No.4: screen shot of solver report

The concentration of Hydrochlorothiazide (C_x), and Losartan (C_y) present in the given formulation sample were found to be 1.98µg/ml, 8µg/ml respectively.

Table No.3 Percentage assay for the three methods

		BLR		CRM		MLS	
	actual Concentration (µg/ml)	Predicted Concentration (µg/ml)	Assay %	Predicted Concentration (µg/ml)	Assay %	Predicted Concentration (µg/ml)	Assay %
HCT	2	1.95	97.5	2.05	102.5	1.98	99
LST	8	8.45	105.6	7.29	91.1	8	100

Acceptance criteria: 85-105%

METHOD VALIDATION: Accuracy

Table No.4: Percentage recovery for all the methods

DRUG	PERCENTAGE	% RECOVERY		
		Bi -linearity	FOR CRM	FOR MLS
ETO	80%	98.66	100.13	99.85
	100%	99.10	99.75	99.72
	120%	99.15	99.84	100.44
THEO	80%	98.89	100.44	99.12
	100%	99.16	100.26	100.50
	120%	99.85	98.98	100.26

Linearity and range:

Table No. 5: Linear equation parameters

Drug	Wave length	For BLRC Method			For Cramer's matrix method(CRM)		
	nm	Linear equation	R^2	RANGE µg/mL	Linear equation	R^2	RANGE µg/mL
HCT	271	y =0.052x-0.023	0.945		y=0.052x-0.023	0.945	
	213	y =0.042x+0.005	0.996	1-5	y=0.042x+0.005	0.996	1-5
LST	271	y=0.052x-0.039	0.964		y=0.052x-0.039	0.964	
	213	y=0.005x-0.0001	0.977	2-10	y=0.005x-0.0001	0.977	2-10

PRECISION

Table No. 6: Percentage RSD for all the methods

DRUG		**Inter day precision (% RSD)**			**Intra day (% RSD)**		
	Concentration	Bi-LRC	CRM	MLS	Bi-LRC	CRM	MLS
HCT	3	1.4	1.8	1.3	1.5	1.6	1.8
	4	1.5	1.6	1.5	1.6	1.7	1.8
LST	6	1.2	1.6	1.4	1.6	1.5	1.8
	8	1.2	1.4	1.3	1.8	1.7	1.6

The proposed spectrophotometric method was found to be linear and the data is presented in the Table No 6. The intra-day and inter-day precision values for both the chemo metric designs were presented in Table No 5 Accuracy was performed in terms of the Percent recovery values and the values for hydrochlorothiazide,losartan by all the chemo metric designs were presented in Table No 4 The assay of the commercial formulation of the drugs were performed and their percentage assay values were presented in Table No 3.

IV. Conclusion

Three simple, accurate, precise, economical methods were developed and validated to estimate hydrochlorothiazide and losartan potassium in bulk dosage form. The developed methods were simple, economic, statistically evaluated and can be utilized for routine analysis in quality control laboratories. Application of chemometric technique helps in complete investigation of data present in the entire data for accurate estimation and minimization of error.

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 Dr.Reddy's laboratories, Pvt. Ltd, Hyderabad for providing the gift samples of the drugs.

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