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Chemometrics assisted UV-spectrophotometric method for simultaneous estimation of aliskiren and hydrochlorothiazide in bulk and their pharmaceutical dosage form

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Abstract: Aim of present research work was to develop and validate chemometrics assisted UV spectrophotometric method for simultaneous estimation of aliskiren and hydrochlorothiazide in bulk and their pharmaceutical dosage form. A five level two factor (2 Drugs) design was applied to get the 25 combinations of mixtures to be prepared. 16 sets of mixtures were used for calibration and 9 set of mixtures were used for validation in wavelength range of 231-334 nm (wavelength interval λ=1 nm) in methanol. Various models like Classical Least Square (CLS), Inverse least squares (ILS), Principal Component Regression (PCR) and Partial Least Square (PLS) were applied to the absorbance data obtained and among which CLS model was found to be best suited. Beer's law was obeyed in concentration range 30 to 150 µg/mL for aliskiren and 2.5 to 12.5 µg/mL for hydrochlorothiazide. The method was then validated according to the International Conference on Harmonization ICH Q2 (R1) and was found to be advantageous in terms of novelty and simplicity.

Keywords: Chemometrics; aliskiren; hydrochlorothiazide; classical least square; inverse least squares; principal component regression; partial least square.

1. Introduction

Chemometrics is a chemical discipline that employs mathematical and statistical tools to design or choose optimal measurement processes and studies.. By evaluating chemical data, it gives the most relevant chemical information [1 In recent years, multicomponent analysis of pharmaceutical mixtures has become more reliant on chemometrics, particularly multivariate calibration techniques [2]. Chemometrics uses two types of data sets: calibration data sets and validation data sets. The concentrations of the analytes in the unknown sample were determined using a calibration data set [3]. Chemometrics quantitative analytical techniques have a wide range of applications and benefits, including the capacity to analyse mixtures for drug determination without the requirement for separation procedures. Chemometric methods have the further benefit of allowing calibration to be performed while ignoring the concentration of all other components except the analyte of interest [4]. When compared to other methods, the chemometrics method can be used to evaluate a large number of samples in a short period of time with greater accuracy and precision [5]. Because analytes' spectra overlap in multicomponent analysis,

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determining analytes becomes challenging. Chemometric approaches are beneficial in this scenario for assessing multiple components at the same time [3,5].

Aliskiren (ALI) is antihypertensive drug which is chemically (*2S,4S,5S,7S*)-5-amino-N-(2-carbamoyl-2-methylpropyl)-4-hydroxy-2-isopropyl-7-[4-methoxy-3-(3-methoxypropoxy)benzyl]-8-methylnonanamide (Figure 1). It is a non-peptide direct renin inhibitor drug. It binds selectively to the catalytic site of renin and competitively block the conversion of angiotensinogen to angiotensin-I so, the chain of Ranin-Angiotensin-Aldosterone system (RAAS) is interrupted. It raises the level of renin in the blood. Aliskiren exerts its antihypertensive activity by binding with high affinity plasma renin and inhibiting the effects of increased renin concentrations as well as the conversion of angiotensinogen to angiotensin I, resulting in decreased plasma renin activity and lower concentrations of angiotensin I, angiotensin II, and aldosterone [6-8].

Hydrochlorothiazide (HCT) is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide (Figure 1). It is a member of the thiazide family of diuretics. It reduces blood pressure by reducing sodium reabsorption in the kidney's distal convoluted tubule. In the nephron, the main site of action is on an electroneutral Na^+-Cl^- co-transporter, where it competes for the chloride site. By blocking sodium transport in the distal convoluted tubule, HCT causes natriuresis and concomitant water loss [6,9].

Several UV spectrophotometric methods [10-34], High performance liquid chromatography (HPLC) methods [35-47], and High-performance thin layer chromatography (HPTLC) methods [48-49] are available for estimating ALI and HCT alone or in combination with other medicines, according to a literature review. There are no chemometrics-assisted spectrophotometric methods available for estimating ALI and HCT simultaneously. The current study's goal was to develop and evaluate a chemometrics-assisted UV spectrophotometric method for simultaneous assessment of ALI and HCT in bulk and pharmaceutical dosage form.

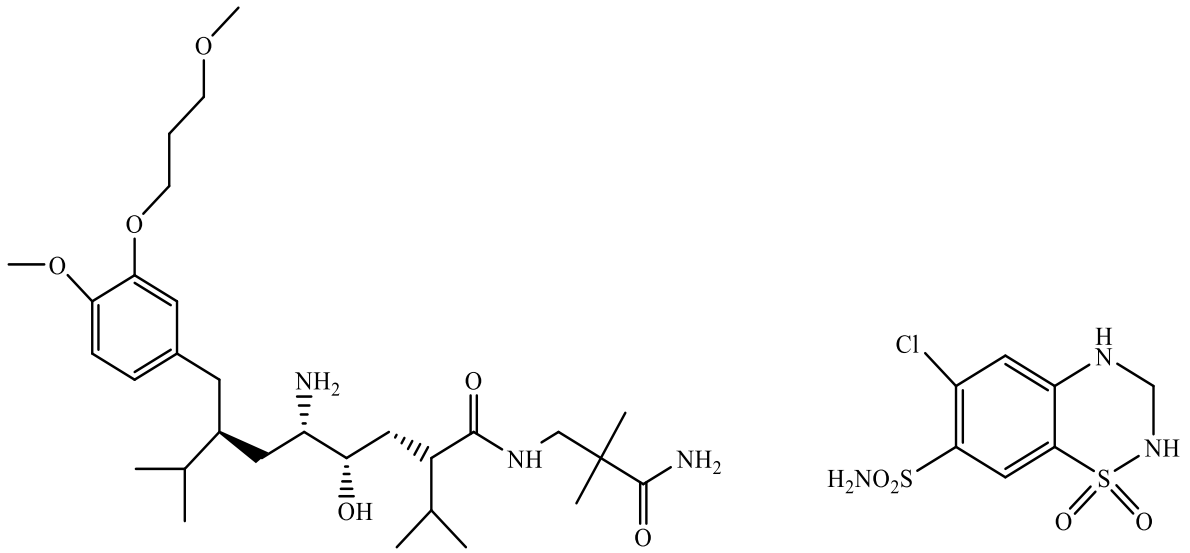

Figure 1. Chemical structure of aliskiren (**1**) hydrochlorothiazide (**2**)

2. Experimental

2.1. Materials and Methods

Equipment used in experiment was Double Beam UV-Spectrophotometer (UV-1800, Shimadzu), Analytical Balance (AUX-220, Shimadzu), and Ultrasonicator (Toshcon, Toshniwal). Solo 8.1 (R8.1.1) software was used for model construction and data analysis. Morepen Laboratories and Dishman

Pharmaceuticals Ltd offered ALI and HCT bulk powders as free samples. All the reagents and chemicals used were of analytical quality and were obtained from Merck India Limited. Purchase Tekturna HCT tablet (300 mg ALI, 25 mg HCT) from a local pharmacy.

2.2. Preparation of Solutions

2.2.1. ALI Standard Stock Solution

50 mg of standard ALI was weighed and transferred to a 50 mL volumetric flask, where it was dissolved in 15 mL of methanol. After sonicating the flask for 15 minutes, the volume was topped up with methanol to generate a stock solution containing 1000 µg/mL ALI.

2.2.2. HCT Standard Stock Solution

2.5 mg of standard HCT was weighed, transferred to a 25 mL volumetric flask, and dissolved in 10 mL methanol. After sonicating the flask for 15 minutes, the volume was brought up to the mark with methanol to yield a solution containing 100 µg/mL HCT.

2.3. Experimental Design

The experimental technique used to construct the calibration set influences the predictive power of the final regression model significantly. To increase the possibility of obtaining higher-quality data from the calibration set, it is critical to select reference solutions with care. The orthogonal design method was used to optimise the calibration model. Because there will be five alternative combinations for each chemical at all concentrations, the design is intended to give a broad coverage of the mixture space. 25 standard samples with various ratios of ALI and HCT (30 to 150 µg/mL for ALI and 2.5 to 12.5 µg/mL for HCT) were generated using a five-level orthogonal array design, so that the concentration of each drug in the resulting solutions was within its own linear dynamic range. The samples were divided into calibration (16 samples) and validation (9 samples) sets based on the Q-residual values obtained for the 25 mixtures. Tables 1 and 2 indicate the calibration and validation combinations employed.

Table 1. Different concentrations of ALI and HCT in calibration set

Sample No.	HCT in µg/mL	ALI in µg/mL
1	2.5	30
2	2.5	150
3	5	30
4	5	60
5	5	120
6	7.5	30
7	7.5	60
8	7.5	90
9	7.5	150
10	10	30
11	10	60
12	10	90
13	12.5	30
14	12.5	60
15	12.5	90
16	12.5	120

Table 2. Different concentrations of ALI and HCT in validation set

Sample No.	HCT in µg/mL	ALI in µg/mL
1	2.5	60
2	2.5	90
3	2.5	120
4	5	90
5	5	150
6	7.5	120
7	10	120
8	10	150
9	12.5	150

The absorbance of the calibration set was measured between 200 and 400 nm, and the spectra were exported to Solo software for further data analysis. At 1 nm intervals, the spectra were digitised. The wavelength range 231-334 showed substantial absorption. As a result, the wavelength range for the current study was chosen at 1 nm intervals. To produce calibration models, the absorption data in the range of 231-334 nm (digitised at 1nm) of 25 laboratory prepared mixtures including ALI and HCT in varying proportions according to the orthogonal array design were subjected to CLS, ILS, PCR, and PLS analysis. 9 mixtures having varying ratios of ALI and HCT were produced for the validation set. The validation set's absorption spectra were acquired over the wavelength range of 231-334 nm. ALI and HCT concentrations were determined using optimised CLS, ILS, PCR, and PLS models. Models that relate the varied spectral intensities from several calibration samples to the known analyte concentrations of the samples were created using multivariate calibrations. These models can be used in multivariate prediction analysis of unknown sample spectra to predict analyte concentrations quickly. Many quality indicators must be evaluated in order to compare the predictive capacity of the models. An estimate of the model's average deviation from the data across all quality measures considered. We can evaluate the model's fit to the calibration data using the root-mean-square error of calibration (RMSEC). It can be calculated using the following formula:

$$RMSEC = \frac{\sqrt{\sum_{i=1}^{n}(\hat{y}_i - yi)^2}}{n}$$

Where $\hat{y}_i$ are the values of the predicted variable after all data are accounted for in the development of the model and n is the number of calibration samples. Root-mean-square error of cross-validation (RMSECV) measures a model's ability to predict data that were not used to develop the model. RMSECV can also be calculated using the same formula as RMSEC, where the $\hat{y}i$ corresponds to predictions for samples other than used in the model formulation.

The Predicted Residual Error Sum of Squares (or PRESS) can be calculated by the equation:

$$RMSECV = \frac{\sqrt{PRESS_k}}{n}$$

where k is the number of model factors (PCs, LVs) and n represents the number of calibration samples. $PRESSk$ represents the total of the squared prediction errors for a model with k factors. The RMSECV and PRESS measurements often refer to cross-validation experiments in which the current calibration data set is divided into separate training and test sets to evaluate the performance of a model constructed utilizing all of the calibration data when applied to new data.

2.4. Method Validation

According to ICH guideline Q2(R1), Accuracy, Intra-day Precision, Inter-Day Precision, and Repeatability were studied as method validation parameters.

2.4.1. Calibration Curve for ALI and HCT

Each standard ALI and HCT stock solution was aliquoted into 10 mL series volumetric flasks in the appropriate volume. The volume of methanol was changed to produce solutions containing 30, 60, 90, 120, and 150 µg/mL ALI and 2.5, 5, 7.5, 10, and 12.5 µg/mL HCT. As stated in Table 1, 16 mixture sets were created. The absorbance at wavelengths ranging from 231-334 nm was measured after sonicating the working standard solutions for 15 minutes.

2.4.2. Recovery

To assess the procedure's accuracy, the usual addition method was utilised to calculate how much ALI and HCT were recovered. Standard solutions of ALI (30, 60, and 90 µg/mL) and HCT (2.5, 5, and 7.5 µg/mL) were added to previously evaluated ALI (60 µg/mL) and HCT (5 µg/mL) sample solutions. The percentage of recovery was calculated by measuring the absorbances and running the concentrations through the CLS model. Each response was calculated by taking the average of three measurements.

2.4.3. Repeatability

The repeatability of ALI and HCT was tested by measuring absorbances of ALI working standard solution (90 µg/mL) and HCT working standard solution (7.5 µg/mL) multiple times (n = 6).

2.4.4. Intermediate Precision

The suggested method's intra-day and inter-day precisions were tested throughout a week by measuring the responses three times on the same day and three times on three different days for three different concentrations of ALI (60, 90, and 120 µg/mL) and HCT (5, 7.5, and 10 µg/mL). The data were presented in terms of relative standard deviation.

2.4.5. Analysis of Marketed Formulation

Twenty tablets were meticulously weighed before being ground into a fine powder. In a 50 mL volumetric flask, a precise amount of powder equal to 50 mg ALI and 4.16 mg HCT was weighed. Sonicate for 15 minutes with 25 mL of methanol. After 5 minutes at room temperature, the sample stock solution containing 1000 µg/mL ALI and 83.2 µg/mL HCT was brought up to the required concentration with methanol. The solution was filtered using Whatman filter paper. The aliquot (4.5 mL) was transferred to a 50 mL volumetric flask, which was then filled with methanol. This resulted in a solution containing 90 µg/mL ALI and 7.5 µg/mL HCT. The solution was then scanned over the wavelength range 231-334 nm, using methanol as a reference, and the absorbance at each 1 nm was recorded.

3. Results and discussion

3.1. Selection of Models

The ILS model has the lowest RMSEC, or calibration error. This implies that the ILS model is likely to best suit the facts. Additionally, the ILS is not the best predictor based on the RMSECV, even for samples inside the original calibration set. The CLS model has the lowest cross-validation error (RMSECV). CLS outperforms the other models in the prediction of fresh samples, with PLS coming in second. The model fit and prediction are most visible in CLS. Calculating PRESS with a cross-validation technique is one way to verify a model's prediction ability. The CLS model has the smallest PRESS value. As a result, was used for this enquiry. The numerous statistical parameters are shown in Table 3.

Table 3. Summary of Statistical Parameters for Chemometrics Models.

Parameter	PLS		PCR		CLS		ILS	
	HCT	ALI	HCT	ALI	HCT	ALI	HCT	ALI
RMSEC	0.065	2.317	0.065	2.317	0.065	2.321	3.46E-14	1.24E-12
RMSECV	0.079	2.851	0.079	2.851	0.078	2.830	0.104	4.469
RMSEP	0.123	1.438	0.124	1.440	0.121	1.370	0.111	4.910
R^2 Cal	1.000	0.997	1.000	0.997	1.000	0.997	1.000	1.000
R^2 CV	0.999	0.995	0.999	0.995	0.999	0.995	0.999	0.989
PRESS	0.099	130.089	0.100	130.096	0.098	128.126	0.172	319.576

3.2. Calibration Curve for ALI and HCT

The concentrations of the prepared calibration sets were predicted, and the Q residuals were obtained. The calibration model data are shown in Table 4. Spectra of ALI (30-150 µg/mL) and HCT (2.5-12.5 µg/mL) are shown in Figure 2 & 4. Linear regression equation, Regression coefficients, Limit of detection (LOD) and Limit of quantification (LOQ) are listed in Table 5.

Table 4. Calibration data of applied method

HCT µg/mL Y 1	ALI µg/mL Y 2	Y 1 Pred.	Y 2 Pred.	Y 1 Res.	Y 2 Res.	Q Res.	Hotelling T^2	KNN Score Distance (k=3)
2.5	30	2.53	30.49	0.0328	0.4860	0.0004	3.684	0.523
2.5	150	2.51	150.81	0.0077	0.8120	0.0001	5.793	0.743
5	30	5.05	28.90	0.0502	-1.1000	0.0002	1.959	0.360
5	60	5.02	60.46	0.0183	0.4570	0.0003	0.916	0.401
5	120	4.96	119.52	-0.0408	-0.4770	0.0009	1.927	0.499
7.5	30	7.44	31.85	-0.0648	1.8500	0.0001	1.071	0.361
7.5	60	7.43	60.15	-0.0741	0.1470	0.0002	0.163	0.347
7.5	90	7.44	91.83	-0.0599	1.8300	0.0009	0.196	0.405
7.5	150	7.56	148.16	0.0600	-1.8400	0.0019	2.990	0.631
10	30	10.15	22.66	0.1520	-7.3400	0.0004	1.857	0.402
10	60	9.94	60.07	-0.0610	0.0720	0.0003	0.396	0.368
10	90	9.94	91.49	-0.0589	1.4900	0.0006	0.423	0.363
12.5	30	12.47	33.98	-0.0349	3.9800	0.0002	2.490	0.380
12.5	60	12.53	60.03	0.0336	0.0321	0.0001	1.732	0.347
12.5	90	12.45	90.64	-0.0540	0.6390	0.0002	1.680	0.352
12.5	120	12.59	118.98	0.0934	-1.0200	0.0005	2.723	0.509

Table 5. Regression characteristics of the method

Drug	**ALI**	**HCT**
Linearity range (µg/mL)	30-150	2.5-12.5
Linearity equation	y = 0.0049x + 0.01	y = 0.0101x + 0.005
LOD (µg/mL)	3.45	0.657
LOQ (µg/mL)	10.67	1.99
Regression coefficient (R^2)	R^2 = 0.9992	R^2 = 0.9990

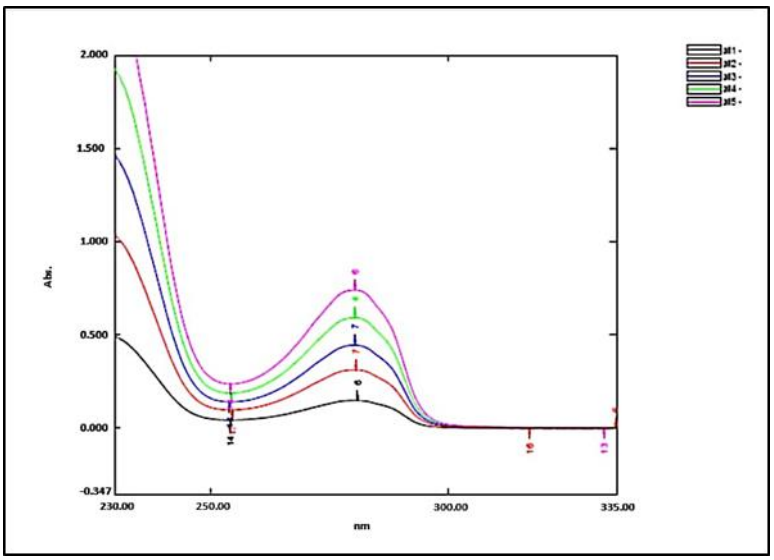

Figure 2. Overlain spectra of ALI (30-150 µg/mL)

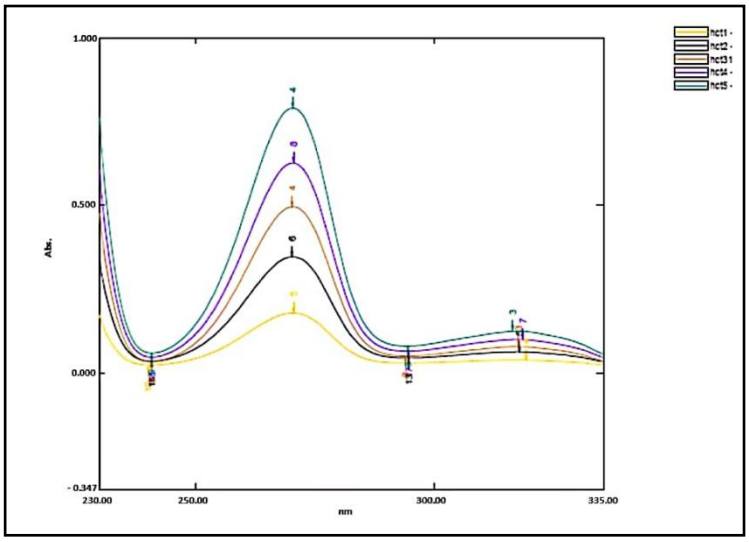

Figure 3. Overlain spectra of HCT (2.5-12.5 µg/mL)

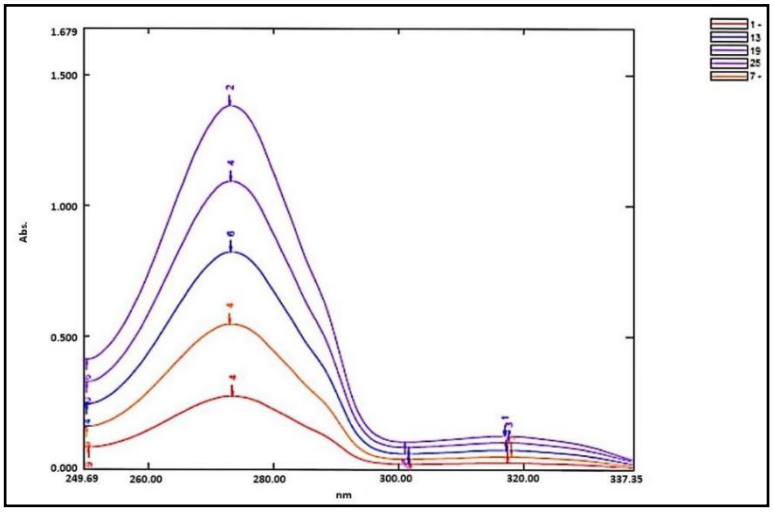

Figure 4. Overlain spectra of ALI (30, 60, 90, 120 and 150 µg/mL) and HCT (2.5, 5, 7.5, 10 and 12.5 µg/mL) sample

3.3. Recovery

The standard addition technique was used to conduct the accuracy investigation. The percent RSD ranges of 1.13-1.45 for ALI and 0.63-1.44 for HCT confirmed the method's precision. The accuracy results are provided in Table 6.

Table 6. Results of recovery study (n=3)

Drug	Conc. (µg/mL)	Standard Added (µg/mL)	Concentration Predicted (µg/mL)	% Recovery ± SD	%RSD
ALI	60	30	91.39	101.54 ± 1.15	1.13
	60	60	120.54	100.45 ± 1.27	1.26
	60	90	150.67	100.44 ± 1.46	1.45
HCT	5	2.5	7.32	97.57 ± 1.41	1.44
	5	5	10.14	101.45 ± 0.96	0.95
	5	7.5	12.67	101.37 ± 0.64	0.63

3.4. Repeatability

The % RSD for repeatability was determined to be 0.851 for ALI and 0.938 for HCT. The results of repeatability are shown in Table 7.

Table 7. Repeatability data of the method

Drug	Conc.	1	2	3	4	5	6	Average	SD	% RSD
ALI	90 µg/mL	91.81	90.67	91.23	91.49	90.64	89.64	90.91	0.773	0.851
HCT	7.5 µg/mL	7.44	7.43	7.36	7.44	7.56	7.38	7.43	0.069	0.938

3.5. Intermediate Precision

The value of % RSD for intra-day precision was found to be in the range of 1.03-1.25 for ALI and 1.04-1.57 for HCT. The inter-day precision was found to be in the range of 1.22-1.66 for ALI and 1.52-1.72 for HCT, which indicated that the method was precise. The results are shown in Table 8 and 9.

Table 8. Intraday precision of the method

Drug	Concentration (µg/mL)	1	2	3	Average	Standard Deviation	%RSD
ALI	60	62.02	60.55	61.17	61.25	0.74	1.20
	90	92.27	91.55	90.41	91.41	0.94	1.03
	120	121.17	119.24	122.21	120.87	1.51	1.25
HCT	5	5.18	5.02	5.11	5.10	0.08	1.57
	7.5	7.45	7.41	7.56	7.47	0.08	1.04
	10	10.24	10.07	10.32	10.21	0.13	1.25

Table 9. Interday precision of the method

Drug	Concentration (µg/mL)	1	2	3	Average	Standard Deviation	%RSD
ALI	60	61.63	62.15	60.19	61.32	1.02	1.66
	90	90.74	92.97	91.71	91.81	1.12	1.22
	120	118.96	122.19	121.57	120.91	1.71	1.41
HCT	5	5.07	5.02	5.19	5.09	0.09	1.72
	7.5	7.53	7.36	7.59	7.49	0.12	1.59
	10	10.29	10.07	10.37	10.24	0.16	1.52

3.6. Analysis of Marketed Formulation

The proposed chemometrics assisted method was successfully applied for determination of drugs in the tablet dosage form. When using the proposed approach, the proportion of ALI and HCT was found to be adequate. Table 10 displays the assay findings in tablet dose form.

Table 10. Assay results of tablet dosage form (n=3)

Brand Name	Drug	Label Claim	% Mean drug found
Tekturna HCT	ALI	300 mg	100.45
	HCT	25 mg	99.25

4. Conclusions

A chemometrics-assisted spectrophotometric approach for determining ALI and HCT simultaneously has been devised and validated. Because of its uniqueness in terms of approach, the method is superior to methods documented in the literature. The method was found to be simple, precise and accurate. Hence, it can be used successfully for the routine analysis of ALI and HCT in bulk and their pharmaceutical dosage forms.

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