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Application of Chromatographic and Spectrophotometric Methods for The Analysis of Aliskiren and Hydrochlorothiazide Antihypertensive Combination

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Abstract

High performance liquid chromatographic (HPLC) and spectrophotometric methods developed for the simultaneous determination of Aliskiren (ALK) and Hydrochlorothiazide (HCT) combination in bulk powder and in tablets dosage form. Determination of ALK and HCT was achieved by chromatographic separation on Econosphere C-18 column using a mobile phase consisting of water (pH 7.5): acetonitrile (50:50) at a flow rate of 0.5 mL.min^{-1} and UV detection at 208 nm. Method validation parameters were found to be acceptable over the concentrations range of 5-150 µg.mL^{-1}, 1-50 µg.mL^{-1} for ALK and HCT respectively. Regarding the spectrophotometric methods, two methods were employed. Simultaneous Equation method, absorbance readings are taken at two wavelengths 277.48 nm (for ALK) and 267.48 nm (for HCT) in methanol. Dual Wavelength method, the difference between absorbance readings are taken at two wavelengths 273.3 nm, 260 nm (for Aliskiren) and 270 nm, 280 nm (for HCT) in methanol. The applied spectrophotometric methods were found to be rapid, specific, precise and accurate over the concentration range of 5 – 150 µg.mL^{-1} and 1 – 41 µg.mL^{-1} for ALK and HCT respectively and can be applied for the routine analysis of these drugs in bulk, and combined dosage form without any interference by the excipients.

Keywords: *High performance liquid chromatography; Simultaneous Equation; Dual Wavelength; Aliskiren; Hydrochlorothiazide.*

1 Introduction

Aliskiren,(2S,4S,5S,7S)-5-amino-N-(2-carbamoyl-2,2-dimethylethyl) – 4 – hydroxyl – 7 - {[4-methoxy – 3 - (3 - methoxypropoxy)phenyl]methyl}-8-methyl-2-(propan-2-yl)nonanamide (Fig. 1a), is an orally active, non-peptide inhibitor of rennin [1]. Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide-1, 1-dioxide (Fig. 1b), is a well-known thiazide diuretic commonly used for the treatment of hypertension [2]. The ALK/HCT is a direct renin inhibitor and thiazide diuretic combination which is used to achieve simultaneous relaxation of blood vessels and removal of excess body fluid, two processes which help to lower blood pressure and help the heart to pump blood more easily [3].

a) b)

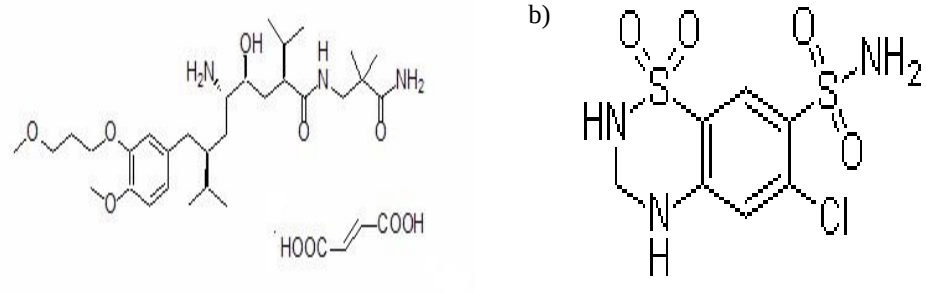


Fig. 1 Structural formula of (a) Aliskiren Hemifurate and (b) Hydrochlorothiazide

Literature survey revealed the estimation of ALK alone or in combination with other anti-hypertensive agents by HPLC [4], RP-HPLC [5-7] and UV-spectrophotometry [8]. Also, HPLC [9-16] , LC-MS[17], UPLC [18] and UV-spectrophotometry [19] methods were reported for the estimation of HCT alone or in combination with other anti-hypertensive agents. Moreover, several analytical methods have been described in literature for the determination of ALK and HCT in pharmaceutical and biological samples. These methods include HPLC [20,21] and MEKC [22].
In the present work, three different, rapid, sensitive and cost-effective methods, namely HPLC, simultaneous equation and dual wavelength spectrphotometry, were described for the simultaneous determination of ALK and HCT in binary mixture and in pharmaceutical dosage form with subsequent validation of all the proposed methods.

2 Experimental

2.1 Instrumentation

- The HPLC system is composed of an Agilent Infinity 1260 series HPLC, 1260 Quat pump VL,1260 ALS Autosampler, 1260 TCC (Column heater) and 1260 VWD VL (UV/Viz detector).
- Econosphere C-18 column (150mm x 4.6mm, 5µm), Grace company(USA).
- Beckman Coulter DU® 800 Series (USA).

2.2 Materials and reagents

All chemicals, solvents and reagents were of analytical or HPLC grade.

- Aliskiren (ALK) (its purity was assessed according to the reported method "spectrophotometrically" and was found to be 99.72±0.47 [8]) and Tekturna tablets (Batch. No: F0025, each 1 tablet labeled to contain 150mg Aliskiren and 12.5 mg Hydrochlothiazide) was kindly supplied by Novartis Co., USA..
- Hydrochlorothiazide (HCT) (its purity was assessed according to official method (potentiometery) and was found to be 99.71 ± 0.44 [23]), was kindly supplied by Sigma Aldrich, USA.
- 5mM Phosphate buffer was kindly supplied by Sigma Aldrich, USA.
- Methanol and acetonitrile were kindly supplied by Sigma Aldrich, USA, of HPLC grade.

2.3 Stock standard solutions

ALK and HCT stock solutions (1 $mg.mL^{-1}$) were prepared by dissolving 100 mg of each standard powder in 100 ml volumetric flask and completed to the mark with methanol.

2.4 Working standard solutions

Twenty ml of ALK and ten ml of HCT respectively, were transferred from the previously prepared standard stock solution of each, into two separate volumetric flasks (100 ml) and the volumes were completed with selected mobile phase for each selected mixture to get final concentrations 200 $\mu g.mL^{-1}$ of ALK and 100 $\mu g.mL^{-1}$ of HCT solutions.

2.5 Laboratory prepared binary mixture

A mixture of 6-144 $\mu g.mL^{-1}$ ALK, 6-12 $\mu g.mL^{-1}$ HCT were prepared.

2.6 Dosage forms solution

Ten Tekturna HCT® tablets were accurately weighed and powdered in a mortar. A quantity of the powdered tablets equivalent to (150 mg) ALK and (12.5 mg) HCT was extracted with methanol (3x20 mL) and filtered using Wattman filter paper no. 40 into a 100 mL volumetric flask. The solution was completed to volume with methanol to obtain a concentration equivalent to 1500 $\mu g.mL^{-1}$ALK and 125 $\mu g.mL^{-1}$HCT.

2.7 The mobile phase

For ALK and HCT mixture, a mixture of water (adjusted to pH 7.5 with Sodium Hydroxide): acetonitrile (50:50) was prepared.

3 Methods

3.1 HPLC method

Accurately measured aliquots of 0.25- 7.5 mL from the 200 µg.mL^{-1} ALK working solution equivalent to 5-150 µg.mL^{-1} ALK and 0.1-5 mL from the 100 µg.mL^{-1} HCT working standard solutions equivalent to1-50 µg.mL^{-1} HCT, were transferred into two series of 10 mL volumetric flasks and the volumes were completed with selected mobile phase. Triplicate 10 µl samples were autoinjected into the selected column (150mm x 4.6mm, 5µm) by autosampler and the chromatograms were recorded using the following chromatographic conditions:

- Mobile phase: water (adjusted to pH 7.5 with sodium hydroxide): acetonitrile (50:50).
- Column: Econosphere C-18 column (150mm x 4.6mm, 5µm).
- Flow rate : 0.5 mL.min^{-1}
- Wavelength : 208 nm

The areas under curve (AUC's) were recorded. Calibration curves were obtained by plotting the area under curve versus the corresponding concentration and the regression equations (1, 2) were computed for ALK and HCT, respectively

$A=16.5411C+19.5225$ $R^2 = 0.9999$ for ALK (1)

$A=58.2766C+45.1845$ $R^2 =0.9999$ for HCT (2)

3.2 For spectrophotometric methods

Accurately measured aliquots of 0.25 - 7.5 mL from the 200 µg.mL^{-1} ALK working solution equivalent to 5-150 µg.mL^{-1} ALK and 0.1-4.1 mL from100 µg.mL^{-1}HCTworking standard solutions equivalent to 1-41 µg.mL^{-1} HCT, were transferred into two sets of volumetric flasks (10 ml) and the volumes were completed with methanol.

3.2.1 Simultaneous Equation method

The absorption spectrum of each solution was recorded over the range of (200-400 nm) against methanol blank. A calibration curve for each compound was obtained by plotting absorbance (A) against concentration (C). The amplitudes at the peak at 277.48 nm & 267.48 nm for ALK and HCT respectively, were used in constructing the calibration curves and the regression equations (3&4) were computed.

$A= 0.0049 C+ 0.0098$ $R^2 = 0.9999$ for ALK (3)

$A= 0.0613 C + 0.0418$ $R^2 = 0.9999$ for HCT (4)

3.2.2 Dual Wavelength method

The absorption spectrum of each solution was recorded over the range of (200-400 nm) against methanol blank. The difference between two absorbencies 273.3 nm and 260 nm for ALK and The difference between two absorbencies 270 nm and 283.3 nm for HCT, respectively were used in constructing the calibration curves which was obtained by plotting the absorbance difference (ΔA) against concentration (C) and the regression equations (5&6) were computed.

$A= 0.0025 C- 0.0005$ $R^2 = 0.9999$ for ALK (5)

$A= 0.0479 C + 0.0356$ $R^2 = 0.9999$ for HCT (6).

The above two spectrophtometric methods were found to be linear over concentration range of 5 - 150 µg.mL^{-1}, 1 - 41 µg.mL^{-1} of ALK and HCT, respectively.

3.3 Simultaneous determination of laboratory prepared mixtures for (ALK/HCT) and (PER/IND) mixtures using the proposed methods

Appropriate dilutions of the previous laboratory prepared mixture were treated as previously mentioned under experimental conditions (2.5) for each method. Regarding the HPLC methods, the concentration of each drug in the laboratory prepared mixture using the corresponding regression equation. Regarding simultaneous equation and dual wavelength the concentration of each drug in the laboratory prepared mixture were calculated using the corresponding principle of each method.

3.4 Simultaneous determination of (ALK/HCT) and (PER/IND) in tablet dosage form using the proposed methods

Appropriate dilutions equivalent to 60 µg.mL^{-1} ALK and 5 µg.mL^{-1} HCT for Tekturna HCT® tablets were transferred from the dosage form solution that previously treated as mentioned under experimental conditions (2.6) for each method,

were transferred into ten 10 mL volumetric flasks were for five of those flasks 0.25, 0.5, 0.75, 1 and 1.25 mL were accurately transferred from the ALK 200 µg.mL^{-1} working solution, equivalent to 5, 10, 15, 20 and 25 µg.mL^{-1} ALK, were for the other five volumetric flasks 0.5, 1, 1.5, 2 and 2.5 mL were accurately transferred from the HCT 100 µg.mL^{-1} working solution, equivalent to 5, 10, 15, 20 and 25 µg.mL^{-1} HCT. Regarding the HPLC methods, the concentration of labeled and added authentic were calculated using the corresponding regression equation. Regarding simultaneous equation and dual wavelength methods the concentration of labeled and added authentic were calculated using the corresponding principle of each method.

4 Results and discussion

4.1 HPLC method

Two chromatographic systems including same C18 column (150mm x 4.6mm, 5µm) and two different mobile phases (acetonitrile: 5mM sodium phosphate buffer pH=7.5 (50:50)) and (acetonitrile: water pH=7.5 (50:50)) values were attempted with flow rate 1 mL.min^{-1}. Perfect separated peaks at time 1.168 min and 1.940 min for ALK and HCT, respectively were obtained with the same resolution for both mobile phases. In-order to delay the retention time of the first peak of (ALK), and to increase the resolution between the two peaks, flow rate was decreased to 0.5 mL.min^{-1} for the selected two mobile phases. Good separation between ALK and HCT with retention time 2.138 min and 3.799 min, respectively, were obtained with same resolution for the two selected mobile phases. Detection was carried out at 208 nm for ALK and HCT, where high detector sensitivity was achieved as ALK shows a sharp distinct peak at this wavelength while HCT shows a wide absorbance range from 200nm to 330nm. Also, slight changes in pH (pH=7.3 and pH=7.7) had no significant effect on the peaks resolution and the retention time. In-order to avoid the common phosphate buffer problems, the mobile phase consisting of acetonitrile: water (adjusted to pH 7.5 with Sodium Hydroxide) (50:50) at a flow rate 0.5 mL.min-1 on Econosphere C-18 column (150mmx4.6mm, 5µm), 208 nm for ALK and HCT was selected (Fig. 2).

A linear relationship was obtained when the AUCs of ALK and HCT were plotted versus the concentration and equations (1, 2) were computed. Method validation parameters were preformed.

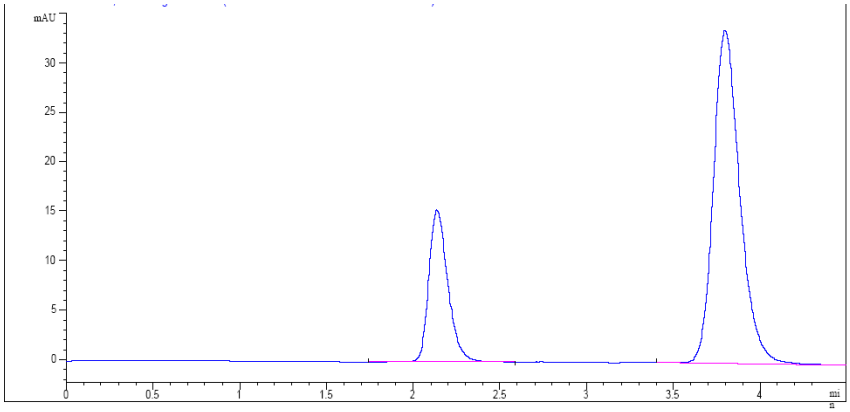


Fig. 2: A typical HPLC chromatogram of 10 µL injector of 10 µg.ml^{-1}Aliskiren and 10 µg.ml^{-1}Hydrochlorothiazide mixture.

The method was successfully applied for the quantitative estimation of ALK and HCT in Tekturna® tablets without any interference from additives. Standard addition technique was also applied. Good recoveries have been obtained for the labeled and added drug (Table 1).

4.2 Spectrophotometric methods

4.2.1 Simultaneous Equation spectrophotometric method

The zero-order spectra of ALK and HCT (Fig. 3) show complete overlapping which prevents the determination of each drug in presence of the other using zero-order spectrophotometry.

In this work, the simultaneous equation method (SE) is presented to solve this problem through measurement of the peak amplitudes at 277.48 nm & 267.48 nm for ALK and HCT, respectively (Fig. 4) and the concentrations in the sample were obtained at the two selected absorption maxima wavelengths in methanol by using the following equations:

$$C_x = \frac{A_1\, ay_2 - A_2\, ay_1}{ax_1\, ay_2 - ax_2\, ay_1} \qquad C_Y = \frac{A_1\, ax_2 - A_2\, ax_1}{ay_1\, ax_2 - ay_2\, ax_1}$$

Where,

A_1=Absorbance of mixture at λ_{max} of ALK (277.48 nm)
A_2=Absorbance of mixture at λ_{max} of HCT (267.48 nm)
ax_1= absorptivity of ALK at λ_{max} of ALK (277.48 nm)
ax_2= absorptivity of ALK at λ_{max} of HCT (267.48 nm)
ay_1= absorptivity of HCT at λ_{max} of ALK (277.48 nm)
ay_2= absorptivity of HCT at λ_{max} of HCT (267.48 nm)

Measurement of the peak amplitudes at 277.48 nm & 267.48 nm for ALK and HCT, respectively were found to be proportional to the concentration of ALK and HCT over a concentration range 5 – 150 and 1- 41 µg.mL^{-1} for ALK and HCT , respectively and the regression equation (3,4) were computed.

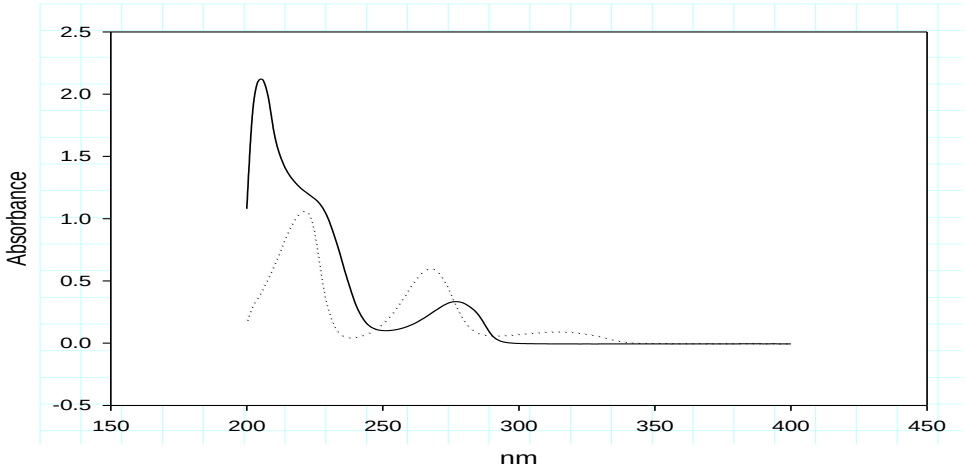
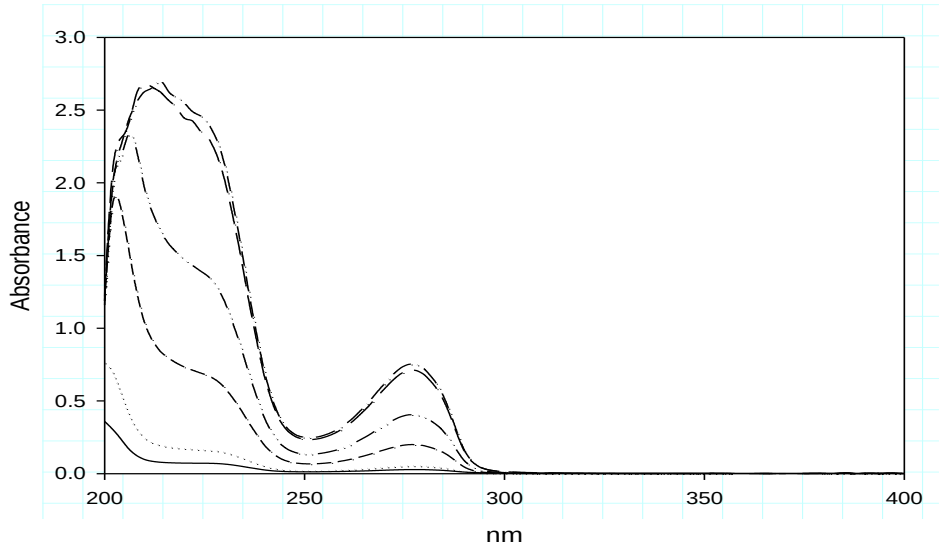


Fig.3: zero-order spectra of (—) ALK and (....) HCT, show complete overlapping.

(a)



(b)

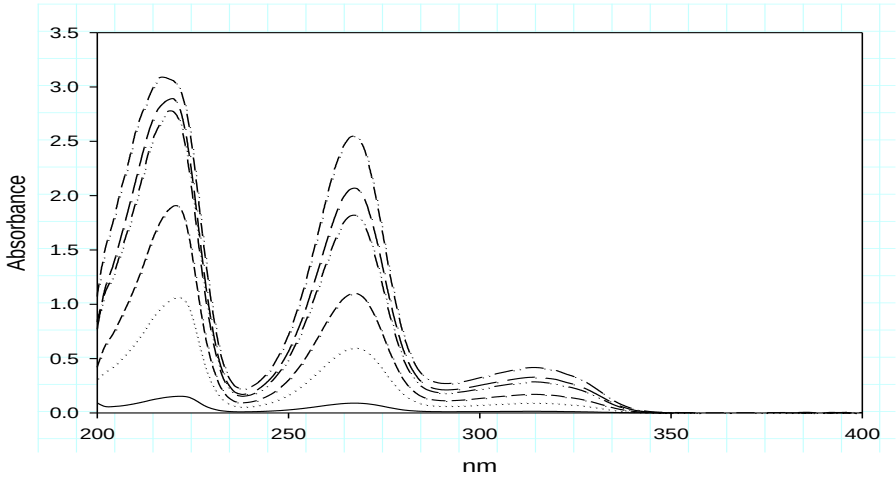


Fig. 4: (a) Aliskirenin methanol (b) Hydrochlorothiazxide in methanol

The method was tested for selectivity by analyzing laboratory prepared mixtures and the mean percentage recoveries of ALK and HCT are 98.97± 0.309&98.69± 0.358, respectively.

The method was successfully applied for the quantitative estimation of ALK and HCT in Tekturna® tablets without interference from additives and the standard addition technique was applied. Good recoveries have been obtained for the labeled and added drug.

4.2.3 Dual Wavelength method

In this work, the Dual wavelength method (DW) is presented to solve the problem of complete overlapping of The zero-order spectra of ALK and HCT, as it was observed that ALK shows the same absorbance at wavelengths of 270 nm and 283.3 nm and HCT shows marked difference of absorbance at these two wavelengths, while HCT shows same absorbance at wavelengths of 273.3 nm and 260 nm and ALK shows marked difference of absorbance at these two wavelengths (Fig. 5). Absorbance difference values were recorded at respective set of two wavelengths and calibration curve was plotted between concentration and absorbance difference values for both drugs and the regression equation (5, 6) were computed.

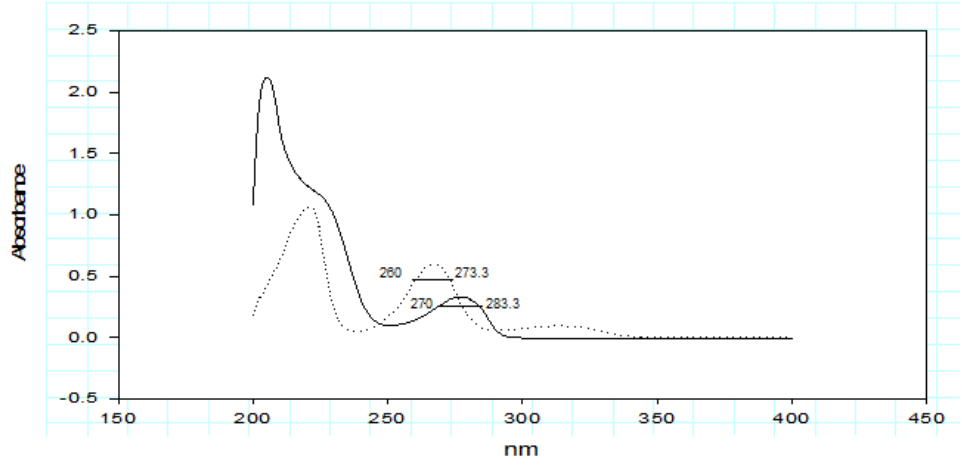


Fig. 5: zero-order spectra of ( ) ALK and (....) HCT.

The method was tested for selectivity by analyzing laboratory prepared mixtures and the mean percentage recoveries of ALK and HCT are 100.00± 0.588&99.39± 0.425, respectively.

The method was successfully applied for the quantitative estimation of ALK and HCT in Tekturna® tablets without interference from additives and the standard addition technique was applied. Good recoveries have been obtained for the labeled and added drug.

5 Method validation

The validation of the methods was assessed by estimation of linearity, accuracy, selectivity, intraday and interday variations ± (RSD) and application of pharmaceutical preparation and further assessed by applying standard addition technique as shown in Table (1).

5. 1 Linearity range

Under the experimental conditions, calibrations for ALK and HCT show linear relationship and regression equations data were shown in Table (1).

5.2 Accuracy

It was determined by applying the proposed methods on at least five different concentrations within the linearity range for drug substance and pharmaceutical dosage forms. The percentage relative standard deviation revealed high accuracy Table (1).

5.3 Precision

For evaluation of the intraday precision, results of three replicate analyses of three different concentrations of ALK and HCT were calculated on a single day. The interday precision was calculated from the freshly prepared samples with the same concentration analyzed on three different days. The percentage relative standard deviations (RSD %) indicating the repeatability and reproducibility of the proposed method Table (1).

5.4 Specificity

Specificity of the proposed methods were tested by the assay of ALK and HCT mixtures in different concentrations and the mean percentage recoveries of each drug was calculated using the corresponding regression equation and the results were listed in Table (1).

5.5 Method validation of dosage forms

The validity of the proposed method was assessed by assay of the pharmaceutical dosage forms and applying the standard addition technique within the linearity range of the three methods and the results were shown in Table(1) revealing the selectivity.

Table1. Analytical and validation data for the determination of ALK and HCT mixture by the proposed methods

Parameters	Methods					
	Simultaneous Equation method		Dual Wavelength method		HPLC	
	ALK	HCT	ALK	HCT	ALK	HCT
Wave length, λ (nm)	277.48	267.48	273.3-260	270-283.3	208	208
Linearity range (µg/ml)	5-150	1-41	5-150	1-41	5-150	1-50
Intercept	0.009	0.0417	-0.0005	0.0356	19.522	45.184
SE of intercept	0.001	0.007	0.0008	0.0074	8.758	10.232
Slope	0.005	0.0613	0.0024	0.0479	16.541	58.276
SE of Slope	1.672E-05	0.0002	1.125E-05	0.0002	0.085	0.334
Regression coefficient (r^2)	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
Residual SS	3.125E-05	0.0004	7.857E-06	0.00039	458.484	819.940
Accuracy [a] (mean ± RSD %)						
Bulk	100.08±0.154	100.01±0.181	99.99±0.207	100.07±0.149	100.22±0.242	100.87±0.111
Dosage Form	100.17±0.688	99.08±0.655	99.63±0.650	99.48±0.660	100.21±0.616	100.72±0.677
Standard addition	99.97±0.209	100.08±0.208	100.18±0.275	100.11±0.297	100.01±0.298	99.96±0.254
Precision [b] (mean± RSD %)						
Inter – day	100.12± 0.324	99.98 ±0.335	99.98±0.313	100.40±0.313	100.45±0.303	100.43± 0.326
Intra – day	99.97± 0.223	100.00±0.197	100.06±0.255	100.22±0.222	100.37±0.236	100.37± 0.289
Specificity	98.97±0.308	98.69±0.358	100.00±0.588	99.39±0.425	100.50±0.561	100.07±0.369

[a] Mean of five different experiments
[b] Mean of nine different experiments

6 Conclusion

The proposed methods are valid, simple and selective and could be used in quality control laboratories for the determination of the cited drugs in drug substance and pharmaceutical product where economy and saving time are essential.

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