A NOVEL STABILITY INDICATING UV SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND CHLORTHALIDONE IN ITS PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

An accurate, precise and simple stability indicating ultraviolet spectroscopic technique was developed to quantify azelnidipine and chlorthalidone, simultaneously was bulk and in combination by absorbance correction method. Ethanol (99.9 %) is used as the solvent in the method. The detection wavelength was found to be 275 nm for chlorthalidone, and 345 nm for azelnidipine. The methodology was validated concerning sensitivity, linearity, reproducibility, accuracy, ruggedness and robustness. Beer-Lamberts law was obeyed in the concentration from 3.2–80 µg mL^{-1} in case of azelnidipine and 5-125 µg mL^{-1} in case of chlorthalidone. Detection limits were obtained as 1.74 µg mL^{-1} for azelnidipine and 2.376 µg mL^{-1} for chlorthalidone. For azelnidipine, quantification limit was 5.272 µg mL^{-1}, while for chlorthalidone it was 7.2 µg mL^{-1}. Accelerated stability studies were carried out. Azelnidipine and chlorthalidone showed different degradation characteristics under acid, alkali, humidity, heats, and oxidized environment.

Keywords: Spectroscopy, azelnidipine, chlorthalidone, stability, validation, ethanol

INTRODUCTION

Hypertension is considered as the major cause of death and "the randomised studies always recommend that reducing blood pressure lowers cardiovascular conditions and death"[1]. One of the major reasons in using combined medication for the control of elevated blood pressure is the difficulty of control with an anti-hypertensive drug. Most of the patients can only attain opposite blood pressure control by availing two or more anti-hypertensive medicines. The goal for FDC development is that by concomitantly administering two comparable drugs in small amounts to produce lesser side effects, potential benefits assignable to synergistic physiological and pharmacological effects can be obtained[2,3]. Combining dihydropyridine calcium channel antagonist azelnidipine (AZE) with a thiazide like diuretic chlorthalidone (CHL), can reduce the blood pressure[4]. Fig. 1 and Fig. 2 shows the chemical structure of azelnidipine, and chlorthalidone, respectively.

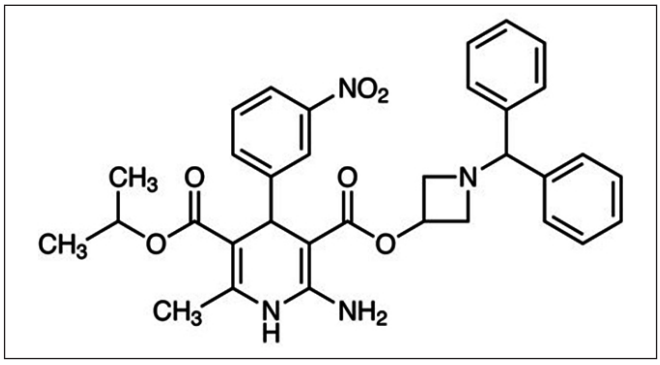

Fig. 1: Chemical structure of AZE

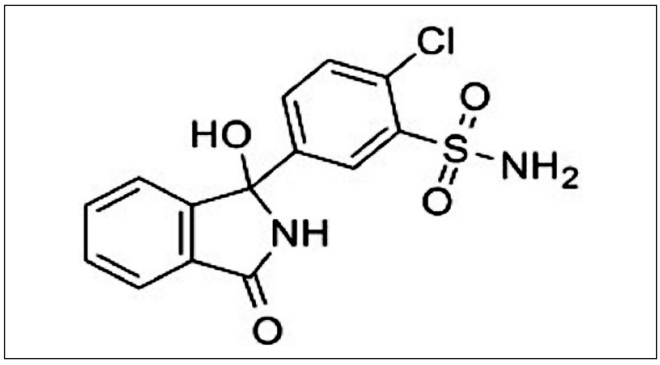

Fig. 2: Chemical structure of CHL

Chemically, AZE is 3-(1-benzhydrylazetidin-3-yl)5-isopropyl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-

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Table I: Assay of formulation

Formulation	Pure drug	Experimental concentration (µg mL^{-1})	Amount found* (mg) (mean ±SD)	% Drug	% RSD
Uniaz CH 8/12.5	Azelnidipine (Label claim 8mg)	41.6	8 ± 0.028	100	0.02
	Chlorthalidone (Label claim 12.5mg)	65	12.5 ± 0.1	100	0.10

**Mean value of three observations*

Table II: Linearity results of azelnidipine and chlorthalidone

Azelnidipine (345 nm)		Chlorthalidone (275 nm)	
Concentration (µg mL^{-1})	Absorbance	Concentration (µg mL^{-1})	Absorbance
3.2	0.037	5	0.033
22.4	0.243	35	0.198
41.6	0.458	65	0.369
60.8	0.66	95	0.529
80	0.887	125	0.693

Table III: Results of accuracy study

Formulation	% Target level	Recovered quantity* (mg) (mean ± SD)	Recovery %	% RSD
Azelnidipine (Label claim 8 mg)	50	8 ± 0.028	100	0.028
	75	8 ± 0.028	100	0.028
	100	8± 0.028	100	0.028
Chlorthalidone (Label claim 12.5 mg)	50	12.5 ± 0.057	100	0.056
	75	12.5 ± 0.057	100	0.057
	100	12.5 ± 0.1	100	0.1

**Mean of three observations*

Table IV: Results of precision study

API	Amount taken (µg mL^{-1})	Intra – day		Inter – day	
		% Content	% RSD	% Content	% RSD
Azelnidipine	41.6	100	0.02	99.9	0.017
		99.8		99.5	
		99.5		99.6	
Chlorthalidone	65	99.5	0.07	99.8	0.05
		100.5		100.1	
		100.8		100.7	

Table V: Robustness results

Drug	Parameter altered	Amount taken (µg mL^{-1})	Amount recovered (mg)	% Drug
AZE	Wavelength (275nm to 285nm)	41.6	7.5 ± 0.028	93.75
CHL		65	12.5 ± 0.057	100

Table VI: Ruggedness results

Drug	Analyst	Quantity taken (µg mL^{-1})	Recovered quantity* (mg) (mean ± SD)	% Drug	% RSD
Azelnidipine	I	41.6	8 ± 0.028	100	0.028
	II		7.95 ± 0.1	99	
	III		8 ± 0.028	100	
Chlorthalidone	I	65	12.5± 0.057	100	0.056
	II		12.6 ± 0.1	100.8	
	III		12.5 ± 0.057	100	

**Mean of three observations*

dihydropyridine-3,5-dicarboxylate"[5]. It is a dihydropyridine calcium channel antagonist. It controls the blood pressure by relaxing the blood vessels and reducing the pressure on them. Trans-membrane Ca^{2+} influx inhibition occurs through smooth muscle's voltage-dependent channels in vascular walls[6–8]. Chemically, CHL is "(*RS*)-2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1-isoindol-1-yl)benzene-1-sulfonamide." It is a diuretic drug. Apart from treating high blood pressure, it is also used to treat diabetes insipidus, renal tubular acidosis, swelling due to liver failure, nephrotic disease and heart failure. It reduces reabsorption of chloride and also sodium by inhibiting the Na^+/Cl^- symporter in distal convoluted tubule. It lowers water quantity in the body by increasing the urine flow, which helps to reduce blood pressure[10-12].

The fixed dose combination of AZE and CHL contains 8 mg of AZE and 12.5 mg of CHL and is available in the market (UNIAZ CH 8/12.5). This combination has additive anti- hypertensive action with fewer side effects.

Literature study revealed that only one UV spectroscopic method[13] was reported for the simultaneous estimation of AZE and CHL. There is no stability indicating work published for this drug combination. So, the current research aimed to develop a cost-effective, accurate, precise, reproducible, rapids stability denoting ultraviolet spectrophotometric technique for estimating AZE and CHL in its pure as well as combined formulation.

Table VII: Results of LOD and LOQ

Drug	Azelnidipine		Chlorthalidone	
	345 nm	275 nm	345 nm	275 nm
LOD (µg mL^{-1})	1.74	2.526	-	2.376
LOQ (µg mL^{-1})	5.272	7.655	-	7.2

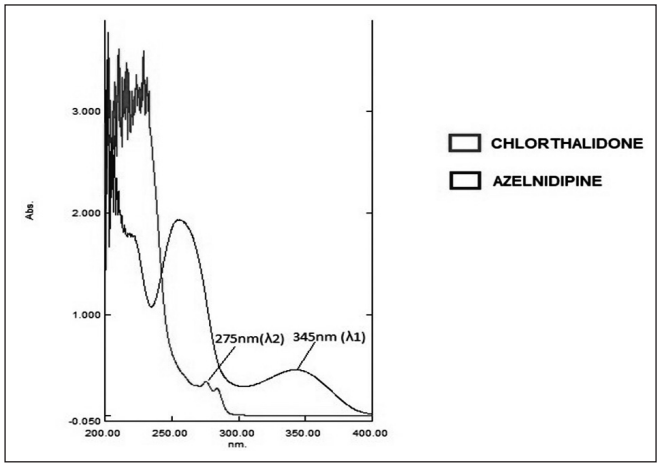

Fig. 3: UV spectra showing detection wavelength of AZE and CHL

MATERIALS AND METHODS

Chemicals

Pure APIs of AZE and CHL were gifted by Rubicon Pharma Ltd., Mumbai and Alembic Pharmaceutical Ltd.,

Table VIII: Degradation study of AZE

SL. No.	Stress type	Stress condition	% Degradation
1	Acid degradation	0.1 N HCL reflux for 30 minutes	56.3 %
2	Base degradation	0.1 N NaOH	-
3	Oxidative degradation	30 % H_2O_2 reflux for 30 minutes	71.9 %
4	Thermal degradation	105 °C for 2 h	7.3 %
5	Photolytic degradation	UV for 24 h	53.9 %
6	Humidity exposure	40 °C and 75 % RH for 8 h	19.9 %

Table IX: Degradation study of CHL

SL. No.	Stress type	Stress condition	% Degradation
1	Acid degradation	0.1 N HCL	25.8 %
2	Base degradation	0.2 N NaOH	-
3	Oxidative degradation	30 % H_2O_2	-
4	Thermal degradation	105 °C for 2 h	1.9 %
5	Photolytic degradation	UV for 24 h	17.9 %
6	Humidity exposure	40 °C and 75 % RH for 8 h	3.6 %

Gujarat, respectively. Uniaz CH 8/12.5, manufactured by Torrent Pharmaceuticals Ltd., with label claim of AZE 8 mg and CHL 12.5 mg was obtained from the local pharmacy. Ethanol (99.9 %) was procured from Technico Laboratory Products, Coimbatore, Tamil Nadu. Hydrogen peroxide, hydrochloric acid and sodium hydroxide were purchased from Spectrum Reagents and Chemicals (P) Ltd., Coimbatore, Tamil Nadu.

Instrumentation

For analytical method development and stability indicating studies of AZE and CHL, a Shimadzu Model Pharmaspec-1800 UV-Visible spectrophotometer (double beam) with software system (UV Probe) was utilized, 1cm matched quartz cell was used. An ultrasonicator by Enertech Electronics Pvt. Ltd., Mumbai was used for the dissolution of the drug in the formulation. For thermal stability studies, a hot air oven manufactured by Technico Laboratory Products Pvt. Ltd., Chennai was used. For photostability studies, a UV chamber by Kemi Lab Equipments, Ernakulam was used.

Preparation of stock and working standard solution

16 mg AZE 25 mg CHL were accurately weighed and transferred into 50 mL separate standard flasks and dissolved in ethanol (99.9 %) and the volume made upto 50 mL to get stock solutions of 320 µg mL^{-1} of AZE and 500 µg mL^{-1} of CHL.

Solutions of 1.3 mL were pipetted out in separate volumetric flask which was further diluted with ethanol. These solutions were mixed well to get concentrations of 41.6 µg mL^{-1} of AZE and 65 µg mL^{-1} of CHL.

Wavelength selection

For selection of detection wavelengths, stock solution of AZE, CHL were scanned in the spectrum mode from 200 nm to 400 nm against ethanol as blank.

Preparation of calibration curve

Serial dilutions of 3.2–80 µg mL^{-1} AZE and 5–125 µg mL^{-1} CHL were prepared into separate 10 mL standard flasks using ethanol. Absorbance of all the solutions of

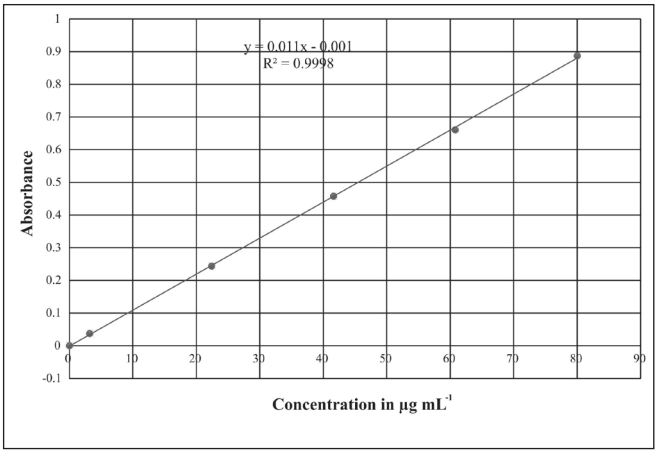

Fig. 4: Linearity graph of AZE at 345 nm

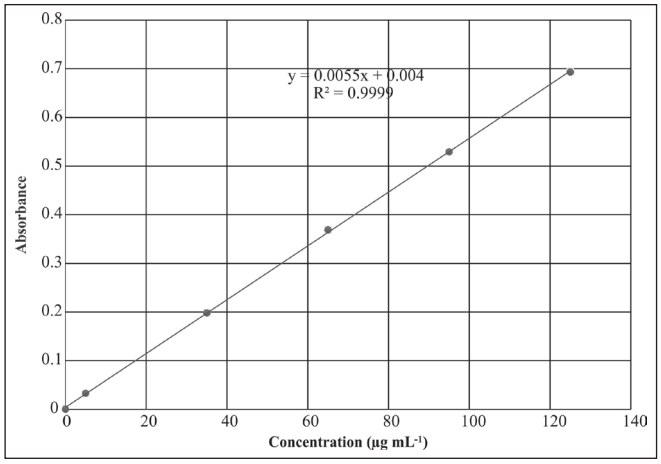

Fig. 5: Linearity graph of CHL at 275 nm

AZE were taken at detection wavelength 345 nm and for CHL were taken at 275 nm. The calibration curves were plotted, and equations for regression analysis were computed.

Assay of tablet formulation

Average weight was calculated by taking weight of 20 tablets. The tablets were finely grounded to a fine powder using mortar and pestle. To 50 mL volumetric flask, weighed quantity corresponding to 8 mg AZE and 12.5 mg CHL were transferred. Ethanol was added for dissolving it, and was ultrasonicated for 15 minutes, finally volume was made with solvent. Using Whatman filter paper (0.45µm pore size), the solution was completely filtered, and the filtrate was collected. 1.3 mL was pipetted out into 10mL standard flask and the volume was made-up using ethanol. The optical density of the resulting solutions was determined at 345nm and 275nm, respectively.

The amount of AZE and CHL in the solution was determined by following formula[14] (Absorbance Correction Method)[15].

"Cx= A1/ax1

Cy= A2 - ax2 cx/ay2"[16].

where,

- *A1, A2 are absorbances of solution (formulation) at 345nm and 275nm, correspondingly*
- *ax1 and ax2 are coefficients of absorptivity for AZE at 345nm and 275nm, correspondingly*
- *ay2, coefficients of absorptivity for CHL at 275nm*

When taking the overlain spectrum of AZE and CHL, it is found that CHL has no absorbance at 345 nm, whereas AZE has good absorbance. Thus, AZE was quantified at 345 nm without interference of CHL by absorption correction method. These two drugs have the absorbance at 275 nm. To quantify CHL, the optical density of AZE was computed at 275 nm using pure drug solution (41.6 µg mL^{-1}). From the total absorbance of the mixture at 275 nm, the absorbance of AZE at 275 nm was deducted. The determined absorbance is the corrected absorbance for CHL. The absorbance of AZE was corrected for interference at 275 nm with the help of absorptivity coefficients at selected wavelengths.

Method validation

As per ICH Q2 (R1) guidelines, the method was validated for different parameters: precision, accuracy, ruggedness, linearity, robustness, detection limit and quantification limit[17].

Linearity

In each 10 mL calibrated volumetric flask, 0.1, 0.7, 1.3, 1.9, 2.5 mL of AZE and CHL was pipetted out separately and then the volume made up with ethanol to obtain concentrations of 3.2–80 µg mL^{-1} AZE and 5–125 µg mL^{-1} CHL. Linearity was measured from the calibration graph.

Accuracy

To the accurately weighed dosage form, 50%, 75% and 100% mixed pure drug was added. This methed was conducted for 3 trials. The percentage recovery and RSD was determined.

Precision

For the study of precision, a concentration of 41.6 µg mL^{-1} AZE and 65 µg mL^{-1} CHL was analysed thrice on successive days, and on the same day and %RSD was determined.

Robustness

The parameter studied by introducing change in the wavelength from 275 nm to 285 nm.

Ruggedness

This parameter was studied by performing the recommended procedure for the combination by three analysts.

LOD and LOQ

With the use of slope, standard deviation of the curve, quantification and detection limits were determined using given formula.

Detection limit = 3.3x(standard deviation/slope)

Quantification limit = 10x(standard deviation/slope)

Stability study[18]

All the stability study procedures were performed by using accurately weighed quantites of AZE (16 mg) and CHL (25 mg).

Acid hydrolysis

Stability samples were taken separately in 50mL standard flasks.10 mL of 0.1 N HCL was added and made up the volume using ethanol. The solutions were refluxed. 1.3 mL of solution was pipetted out and added to 10 mL standard flask. The volume was made up with the ethanol and scanned over 200 nm to 400 nm.

Base hydrolysis

Samples were transferred separately into two 50 mL standard flask. 10 mL of 0.1 N NaOH was added and made up the volume with ethanol. The solutions were refluxed. 1.3 mL of solution were pipette out and added to 10 mL standard flask. The volume was made up with ethanol and scanned over the 200 nm to 400 nm range

Oxidative hydrolysis

Samples were transferred separately into two 50 mL standard flasks. Added 10 mL of 30 % H_2O_2 and made up the volume with ethanol. The solutions were refluxed. 1.3 mL of solution was pipetted and added to 10 mL standard flask. Volume was made up with ethanol and scanned over 200 nm to 400 nm.

Thermal degradation

Weighed samples were placed in separate petri dishes and kept in hot air oven for 3 h at 105 °C. Then, they were transferred into two separate, 50 mL volumetric flask and diluted using ethanol. 1.3 mL of each solution was pipetted and added to 10 mL standard flask. The volume was made up with ethanol and scanned over 200 nm to 400 nm.

Photolytic degradation

Samples were transferred separately into two, 50 mL standard flasks and diluted using ethanol. It was placed in UV chamber (shorter wavelength) for 24 h, 1.3 mL of solution was pipetted out, and added to 10 mL standard flask. The volume was made up with ethanol, and scanned over 200 nm to 400 nm.

Humidity exposure

Weighed samples were placed in separate petri dishes and kept in humidity chamber at 40 °C and 75 % RH for 8 h, 1.3 mL of solution were pipetted, and added to 10 mL standard flask. The volume was made up with ethanol and scanned over 200 nm to 400 nm.

RESULTS AND DISCUSSION

Wavelength selection

AZE and CHL stock solutions were prepared separately, and the absorbances were noted at 345 nm for AZE and 275 nm for CHL against ethanol as blank. The spectra of the drugs are given in Fig. 3.

Assay of tablet formulation

Contents of AZE and CHL in marketed product determined by the new method, and are given in Table I.

METHOD VALIDATION

Linearity

The AZE and CHL obeyed Beer-Lambert law in ethanol. The range of concentration of AZE was found to be 3.2 to 80 µg mL^{-1} with R^2 of 0.9998, and CHL was found to be 5 to 125 µg mL^{-1} with regression 0.9999, as shown in Figs. 4, 5 and Table II.

Study of accuracy

Proposed method's accuracy was studied at 50%, 75% and 100% percentage levels. Recovery percentage and relative standard deviation were within the limits. Results are given in Table III.

Precision

Triplicate analysis was carried out using the proposed method during the day and on three successive days. Results are within the limits as shown in Table IV.

Robustness

Robustness was determined by variance of wavelength from 275 nm to 285 nm and the effects in the results were recorded. The proposed method was robust. Results are given in Table V.

Ruggedness

Ruggedness study data are given in Table VI.

LOD and LOQ

The lowest detection and quantification limits were computed, and results are shown in Table VII. The sensitivity of the method is indicated by the obtained values.

Stability indicating studies

Acid hydrolysis

CHL undergoes acid degradation, suddenly after adding 10 mL of 0.1 M HCL. 25.8 % degradation of CHL and in case of AZE, 56.3 % degradation was observed.

Base hydrolysis

A major degradation was noticed in base hydrolysis in which AZE and CHL were degraded immediately after the addition of the base. In case of both the drugs, the peaks were not detected.

Oxidative degradation

CHL was degraded immediately after the addition of 30 % hydrogen peroxide, whereas AZE degraded significantly.

Thermal degradation

Thermal degradation of AZE and CHL was studied. CHL showed only 1.9 % degradation when kept in hot air oven at 105 ^{o}C for 2 h. In case, of AZE 7.3 % degradation was observed.

Photolytic degradation

Stability of AZE and CHL under UV light was studied. AZE showed 53.9 % degradation after exposure at 254 nm for 24 h. CHL showed a certain stability towards photolytic condition. Only 17.9 % was degraded under the same conditions.

Humidity exposure

In this study, CHL showed relatively good stability to humidity condition. Only 3.6 % degradation occurred after 8 h. AZE was found to show 19.9 % degradation after 8 h.

During the accelerated stability studies, it was observed that a major degradation occurred in base hydrolysis in which AZE and CHL were degraded immediately after the addition of the base. AZE degraded significantly under acid hydrolysis and oxidative degradation, moderately under photolytic and thermal stress condition and degraded the least under humidity exposure. CHL degraded significantly under acid hydrolysis and oxidative degradation, moderately under photolytic and the least under thermal and humidity exposure. The results are shown in Table VIII and IX.

CONCLUSION

A novel validated stability indicating UV spectroscopic method for quantification of azelnidipine and chlorthalidone in FDC was developed. The investigational results indicate that the spectroscopic technique is simple, accurate, specific, robust, precise and rugged. So, the developed procedure can be utilized for regular formulation analysis. The developed method will be helpful in providing storage recommendations.

REFERENCES

1. Rowan C. G., Turner J. R., Shah A. and Spaeder J. A.: Anti-hypertensive treatment and blood pressure control relative to hypertension treatment guidelines. **Pharmacoepidemiol. Drug Saf.**, 2014, 23(12), 1294-1302.
2. Ram C. V.: Fixed-dose triple-combination treatments in the management of hypertension. **Managed Care (Langhorne, Pa.).,** 2013, 22(12), 45-55.
3. Chrysant S. G.: Using fixed-dose combination therapies to achieve blood pressure goals. **Clin. Drug Investig.**, 2012, 28(11), 713-734.
4. Wan X., Ma P. and Zhang X.: A promising choice in hypertension treatment: Fixed-dose combinations. **AJPS.,** 2014, 9(1), 1-7.
5. Indian Pharmacopeia, The Indian Pharmacopoeia Commission, Ghaziabad. 2018, pp. 1304-1305.
6. https://en.wikipedia.org/wiki/Azelnidipine (Accessed on 20-Aug-2022).
7. https://www.drugbank.ca/drugs/DB09230 (Accessed on 22-Aug-2022).

8. https://pubchem.ncbi.nlm.nih.gov/compound/Azelnidipine (Accessed on 24-Aug-2022).
9. Indian Pharmacopeia, The Indian Pharmacopoeia Commission, Ghaziabad 2018, pp. 1603-1604.
10. https://en.wikipedia.org/wiki/Chlorthalidone (Accessed on 02-Sep-2022).
11. https://www.drugbank.ca/drugs/DB00310 (Accessed on 05-Sep-2022).
12. https://pubchem.ncbi.nlm.nih.gov/compound/Chlorthalidone (Accessed on 06-sep-2022).
13. Mahesh A., Muhammad S. C., Venugopala K. N., Anroop B. N., Sreeharsha N., Sheeba S., Marysheela D., Abdulmalek A. B., Abdulrahman I. A., Efren P. M. and Pran K. D.: Mathematically processed UV spectroscopic method for quantification of chorthalidone and azelnidipine in bulk and formulation: evaluation of greenness and whiteness. **Int. J. Spectrosc.,** 2022, 05, 1-13.
14. Rahul K., Megha G., Aneez P. M. and Jeeva A. S.: Development and validation of a new simple and rapid UV spectroscopic method for cefalexin and potassium-clavulanate in pure and dosage form. **IJSR.,** 2022, 11(9), 917-920.
15. Beckett A. H. and Stenlake J. B., Practical pharmaceutical chemistry, (4th ed). India: CBS; 2007.
16. Rekha Y., Haribabu Y., Velayudhankutty S., Eapen S. C. and Jane M.: UV spectrophotometric absorption correction method for the simultaneous estimation of efavirenz, lamivudine and zidovudine in tablet dosage forms. **Pharma Innov. J.,** 2013, 03, 2174.
17. ICH Harmonised Tripartite Guideline, validation of analytical procedures: text and methodology Q2(R1) current step 4 version, November (2005).
18. ICH Harmonised Tripartite Guideline, stability testing of new drug substances and products Q1A(R2), ICH, Geneva, Switzerland, (2003).

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