Research Article

Applicability of Validated Spectrophotometric Technique for the Simultaneous Estimation of Combined Dosage Forms of Levocetirizine Di HCl with Ambroxol HCl and Phenylephrine HCl

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ABSTRACT

A simple, precise and accurate spectrophotometric method was developed for simultaneous estimation of LevoCetirizine DiHCl with Ambroxol HCl and phenylephrine HCl in the tablet dosage form. This method was based on use of Vierordt's method and absorption correction method for estimation of both the drugs from tablet, readily available distilled water was used as solvent. Ambroxol HCl (ARX), LevoCetirizine DiHCl (LCZ) and phenylephrine HCl (PER) showed maximum absorbance at 244, 231 and 273nm respectively. All the three drugs were followed Beer,s law in the concentration range 5-50µg/ml (ARX), 5-25µg/ml (LCZ) and 5-60µg/ml (PER). Assay of tablet was carried out and results were found 99.96% and 99.39% respectively for ARX and LCZ content dosage form while 101.23% and 99.95% respectively for LCZ and PER content dosage form. Accuracy's SD data were varied from 1.5167-3.6215 for ARX and 1.8818-3.8305 for LCZ and from 1.1938-2.2121 for LCZ and 0.7698-2.0016 of PER. Precision study results were found within acceptable limit. The suitability of this method for estimation of both drugs was proved by validation. Statistical analysis data shows that the developed method is sound under analytical condition and can be used for routine analysis of both drugs.

Keywords: Ambroxol HCl (ARX), LevoCetirizine DiHCl (LCZ), Phenylephrine HCl (PER), Vierordt's method, absorption correction method, Ecofriendly.

INTRODUCTION

The primary target of development of this technique was to achieve concurrent detection of levocetirizine and its combination with ambroxol and phenylephrine under common conditions that are applicable for frequent quality control of dosage form in laboratories[1].

A new approach in the research field has significant impact[2] viz. design of modified technique on new modern instruments. Ambroxol HCl (ARX) chemically is trans-4-[(2-amino-3,5 dibromobenzyl) amino]-cyclohexanol Hydrochloride[3, 4]. Ambroxol is a metabolite of bromhexine. A mucolytic expectorant is a derivative of alkaloid vasicine, also naturally obtains from *Adhatoda vasica*, a potent mucolytic agent capable of inducing thin copious bronchial secretion. It is given in a usual oral daily dose of 60 to 120 mg of the HCl in 2 divided doses [5-7].

Literature survey reveals that there are reported methods for estimation of ARX alone or with other drugs includes lonely UV spectroscopic method [8], with other drug UV spectroscopic method [9-11], lonely HPLC method [12] , with other drug HPLC methods [13-15], stability indicating HPLC technique [16], impurity detection HPLC method [17], HPTLC technique [18], stability indicating HPTLC method [19], bio analytical HPTLC [20], LC-MS/MS bio analytical [21], capillary gas liquid chromatography method [22], fluorometric detection method [23].

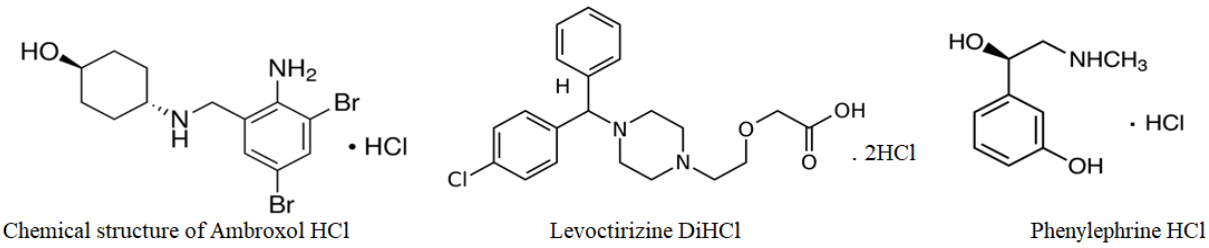
Levocetirizine DiHCl (LCZ) is a second generation antihistaminic agent and chemically it is R-2-[2-[4-[(4-chlorophenyl) phenyl methyl] piperazin-1-yl]ethoxy]acetic acid dihydrochloride [3, 4]. This R enantiomer has a 30 fold higher affinity than the S enantiomer and dissociates more slowly from H1 receptors. Levocetirizine is indicated for the relief of symptoms associated with allergic rhinitis in adults and children 6 years of age and older; in adult dose 2.5–5 mg is adequate. Levocetirizine is the acid metabolite from oxidation of the primary alcohol of the antihistamine hydroxyzine with marked affinity for peripheral H_1 receptor. It is also used in chronic urticarial [5-7].

Reported methods for estimation of LCZ alone or with other drugs in literature survey were found includes lonely UV spectroscopic method [24, 25], with other drug UV spectroscopic method [26, 27], lonely HPLC method [28-30] , with other drug HPLC methods [31-33], stability indicating HPLC technique [34], QbD based HPLC method [35], bioanalytical HPLC technique [36], synchronous fluorometric detection method [37]. stability indicating HPLC method [38].

Phenylephrine hydrochloride (PER) chemically is (R)-1-3-Hydroxy phenyl)-2-methyl amino ethanol hydrochloride [3, 4] is selective direct acting α adrenoceptor agonist differs from epinephrine only in lacking a *p* OH group. It is direct acting sympathomimetic agent with mainly direct effects on adrenergic receptors [5]. It has mainly alpha adrenergic activity used for the symptomatic relief of nasal congestion (mydriatic decongestant) in 1% conc in both oral and topical preparations. Oral dose of 10 mg to maximum 60 mg daily is prescribed by physicians. When applied to mucous membrane it reduces congestion and swelling by constricting the blood vessels of the membrane [6, 7].

Described methods for estimation of PER alone or with other drugs in literatures found that includes lonely stability UV spectroscopic method [39], with other drug UV spectroscopic method [40, 41], lonely UV spectroscopic technique [42] , with other drug stability indicating HPLC methods [43], HPLC technique [44, 45], chemometric HPLC technique [46], HPTLC method [47] were reviewed.

All three drugs are official in Indian and British pharmacopoeias [3, 48]. The proposed method is validated as per ICH guidelines. The chemical structure of drug molecule is shown in Fig No 1.



Chemical structure of Ambroxol HCl | Levoctirizine DiHCl | Phenylephrine HCl

Figure 1: Chemical structure of drug molecules

Instrument

Analysis was performed with a UV-1900i Shimadzu Double beam spectrophotometer (Shimadzu, Kyoto, Japan) with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with 10 mm matched Quartz cells was used. Drugs were weighed on electronic balance 'Afcoset' (The Bombay Burmah Trading corpo Ltd) with accuracy ± 0.1 mg Model No. ER 200A and Digital Ultrasonic cleaner 1.8 Ltr (Labman scientific Instruments Chennai) was used for degassing the solutions.

Pure Drugs and Lab Reagents

Gift samples of Ambroxol HCl from BLD pharmatech Co Hyderabad, LevoCetirizine DiHCl from Akums Drugs and Pharmaceuticals Ltd Haridwar Uttarakhand and Phenylephrine HCl from Cure Medicines Pvt Ltd. Pune, Maharashtra, India were procured. Double distilled water freshly prepared in laboratory was used. Formulation containing Ambroxol HCl 60 mg and LevoCetirizine DiHCl 5 mg, Brand Name Levocold (TTK Healthcare Ltd) dosage form containing Levocetrizine diHCl 5 mg and Phenylephrine HCl 10 mg Brand Name Levocet-D plus (Hetero Drugs Ltd) were procured from local market.

Selection of solvent

ARX is soluble in water, methanol and practically insoluble in dichloromethane [7], methylene chloride [48].LCZ is freely soluble in water, methanol [7] practically insoluble in methylene chloride and acetone [48]. PER is freely soluble in water, alcohol sparingly soluble in methanol and dissolves in dilute mineral acids [4, 48].

Preparation of Standard stock solution

Accurately weighed each pure drug powder equivalent to 25 mg of each of Ambroxol HCl, LevoCetirizine DiHCL and Phenylephrine HCl separately and transferred into separate 100 ml volumetric flask. Dissolved in water and volume was made up to 100 ml with water which produced 250mcg/ml conc of each analyte. Further aliquot of stock solution was diluted to obtain 20µg/ml of each drugs standard solution.

Selection of Wavelength and method

Each Prepared Standard solutions of ARX, LCZ and PER were scanned in the spectrum mode from 400 nm to 200 nm. From UV spectra (Fig No 2 and 3) it was found that ARX has measurable absorbance at 244 nm (λ_{max}) with interference by LCZ of constant absoptivity; similarly LCZ was shown maximum absorbance at 231 nm (λ_{max}) and less interference by PER which has peak absorbance at 273 (λ_{max}). Overlaid spectra of ARX and LCZ directed applicability of simultaneous equation method [49-51] and spectra of LCZ and PER were guided usefulness of absorption correction method [49-51].

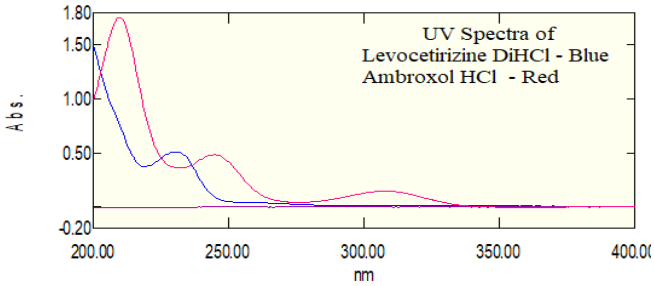


Figure 2: UV overlain spectra of Ambroxol HCl and Levocetirizine DiHCL

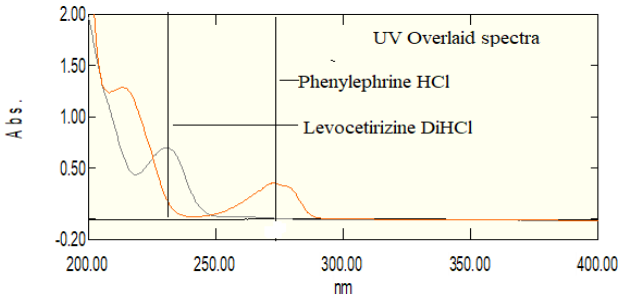


Figure 3: UV overlain spectra of Phenylephrine HCl and Levocetirizine DiHCL

The wavelength 273nm was comfortable for exclusive detection of PER where LCZ shows negligible or zero interference; directed applicability of absorbance correction method. The method was developed and validated as per guidelines directed and described in ICH [52, 53].

Estimation of Ambroxol hydrochloride and Levocetirizine dihydrochloride

Simultaneous equation method

Standard solutions each of 20µg/ml of Ambroxol HCL and LevoCetirizine diHCL were prepared in 10 ml volumetric flask and scanned in 200 to 400 nm wavelength range against water as blank. Absorption spectra of both drugs were recorded and the λ_{max} of Ambroxol HCl and Levocetirizine diHCl was found at 244 and 231nm respectively. From overlain spectra both drug has absorption at these two wavelengths hence to overcome the interference simultaneous equation method was applied. 231 nm wavelengths selected as λ_1 and 244 nm wavelength selected as λ_2. To calculate conc of each drug at wavelength the formula derived from equation as

$$C_x = \frac{A_2 . a_{y_1} - A_1 . a_{y_2}}{a_{x_2} . a_{y_1} - a_{x_1} . a_{y_2}}$$

$$C_y = \frac{A_1 . a_{x_2} - A_2 . a_{x_1}}{a_{y_1} . a_{x_2} - a_{y_2} . a_{x_1}}$$

Where

C_X and C_Y are conc of LevoCetirizine diHCl and Ambroxol HCl respectively

ay_1 and ay_2 are absorptivities of Ambroxol HCl at 231 and 244 nm respectively

ax_1 and ax_2 are absorptivities of LevoCetirizine diHCl at 231 and 244 nm respectively

A_1 and A_2 are absorbances of Tablet solution at 231 and 244 nm respectively.

Estimation of Levocetirizine dihydrochloride and Phenylephrine hydrochloride

Absorption correction method

Standard solution each of 15µg/ml of LCZ and 30µg/ml PER were prepared in 10 ml volumetric flask and scanned in 200 to 400 nm wavelength range against water as blank. Absorption spectra of both drugs were recorded and the λ_{max} of LCZ and PER was found at 231 and 273nm respectively. From overlain spectra it was found that LCZ shows almost zero absorbance at 273 nm hence this wavelength is considered for exclusive detection of PER; and at 231nm wavelength the interference of PER was accounted by absorption correction method. To calculate conc of each drug from mixture the formula derived from equation as

$$C_y = \frac{A_s}{ay_2} \qquad C_x = \frac{A_s - a_{y_1} . C_y}{a_{x_1}}$$

Where

A_s = absorbance at 231and 273nm for cx and cy detection respectively of sample containing PER and LCZ

a_{X1} = absorptivity of LCZ at 231nm

a_{Y1} = absorptivity of PER at 231nm

a_{Y2} = absorptivity of PER at 273nm

C_X and C_Y are conc of LCZ and PER respectively

Analysis of Tablet Formulation of ARX and LCZ

Twenty tablets were weighed and crushed to powder, powder equivalent to 60 mg of AMB and 5 mg of CET were weighed and transferred to 100 ml volumetric flask, powder was dissolved in water and volume was made upto 100 ml with water. Mixed well, Resulting solution was filtered through whatman filter paper. Aliquots of solution were diluted to 10 ml into 10 ml volumetric flask and solution was scanned in 200 to 400nm wavelength range. Absorption of solution was measured at 231 and 244 nm Fig.No.2. Amount of each drug in solution was calculated by the method and results are tabulated.

Analysis of Tablet Formulation of LCZ and PER

Twenty tablets were weighed and crushed to powder, powder equivalent to 10 mg of PER and 5 mg of LCZ were weighed and transferred to 100 ml volumetric flask, powder was dissolved in water and volume was made up to 100 ml with water. Mixed well, Resulting solution was filtered through whatman filter paper. Aliquots of solution were diluted to 10 ml into 10 ml volumetric flask and solution was scanned and absorption of solution was measured at 231 and 273 nm. Fig No.3 Amount of each drug in solution was calculated. The method was validated as per ICH guidelines [52, 53].

Linearity

The linearity of an analytical method is its ability to obtain test results which are directly proportional to the conc of analyte. Series of standard solutions were prepared in conc range of 5 to 30µg/ml for LCZ, 5 to 50µg/ml for ARX and 5 to 60µg/ml for PER and scanned in 200 to 400 nm range in spectrum mode of the spectrophotometer. Absorbances were recorded at 231, 244 and 273 nm wavelength for LCZ, ARX and PER respectively and calibration graph was plotted in Microsoft office excel software tool to obtain the standard regression curve and its analysis as slope, intercept, and correlation coefficient.

Precision

The precision study was carried out by performing assay of tablet six times. Also the reproducibility in result was studied by interday and intraday precision. SD and RSD were calculated to show precision of the method.

Accuracy

The accuracy of an analytical method expresses the closeness of an agreement between test result and true result. Accuracy study was performed by recovery study i.e. standard addition method. Diluted standard solutions of LCZ, ARX and PER were prepared and standard solutions added in 80,100 and 120% proportionate. Three replicates at each of these three levels were prepared and measured and % of conc, SD and RSD were calculated.

Robustness and ruggedness

It is measure of capacity of analytical procedure to remain unaffected by small but deliberate variations in method parameter.

Limit of detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of LCZ, ARX and PER of the proposed method were determined from the calibration curve method and calculated as 3.3σ/s and 10σ/s for LOD and LOQ respectively; σ is the standard deviation of calibration curve and s is the slope of regression line.

RESULTS AND DISCUSSION

Method development comprises numerous steps; of which solvent selection, method for measurement selection are significant one. Uses of eco-friendly solvents have got remarkable weightage due to low cost, readily available and environmentally sound. Drugs underlying analysis must have appreciable solubility in the selected solvent. Solubility of LCZ, ARX and PER was studied in each solvent; and in distilled water all drugs were shown more and appreciable absorbance as compare to other solvent. Optical parameters were given in Table 1.

Table 1: Selected critical parameter for analytical method of LCZ, ARX and PER

Parameter	Selected variables For		
	LCZ	ARX	PER
Wavelength range	400-200 nm	400-200 nm	400-200 nm
Wavelength	231 nm	244 nm	273 nm
Solvent	Distilled water	Distilled water	Distilled water
Scan speed	Fast	Fast	Fast
Sampling interval	± 0.2 nm	± 0.2 nm	± 0.2 nm

System Suitability

The absorbances of six replicates of standard solutions (20µg/ml) are reported in Table 2. The SD of LCZ, ARX and PER was found within acceptable limit and meets the system suitability requirements indicates method was suitable for analysis.

Table 2: Absorbance of drugs

S. N.	Conc in µg /ml	Absorbance of LCZ	Absorbance of ARX	Absorbance of PER
1	20µg /ml	0.731	0.492	0.221
2	20µg /ml	0.749	0.476	0.232
3	20µg /ml	0.738	0.484	0.246
4	20µg /ml	0.733	0.473	0.219
5	20µg /ml	0.724	0.489	0.248
6	20µg /ml	0.741	0.482	0.234
	SD	0.008671	0.007312	0.01212

Linearity

The overlay spectra obtained in linearity study was shown in Fig No 4, 5 and 6 and the obtained calibration curve of all these analytes was found to be linear in the selected conc range as shown in Fig No 7. The regression equation of line and its parameters slope, r^2 value and intercept are tabulated in Table No 3, which proved the linear relationship between conc and obtained response.

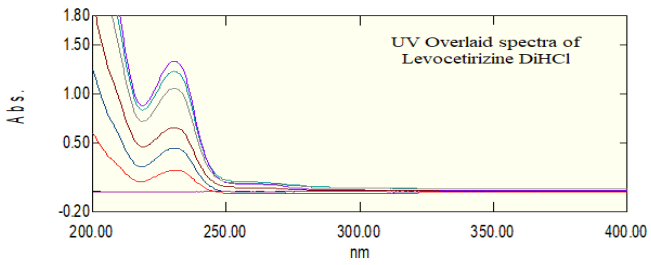


Figure 4: Linearity study of Levocetirizine DiHCl

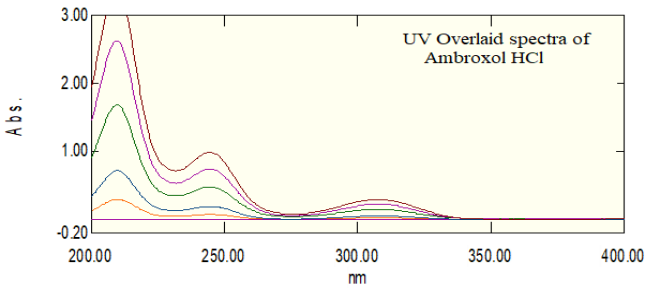


Figure 5: Linearity study of Ambroxol HCl

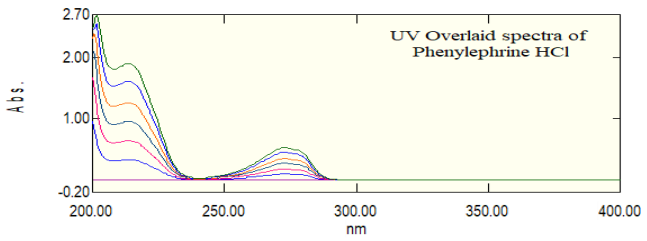


Figure 6: Linearity study of Phenylephrine HCl

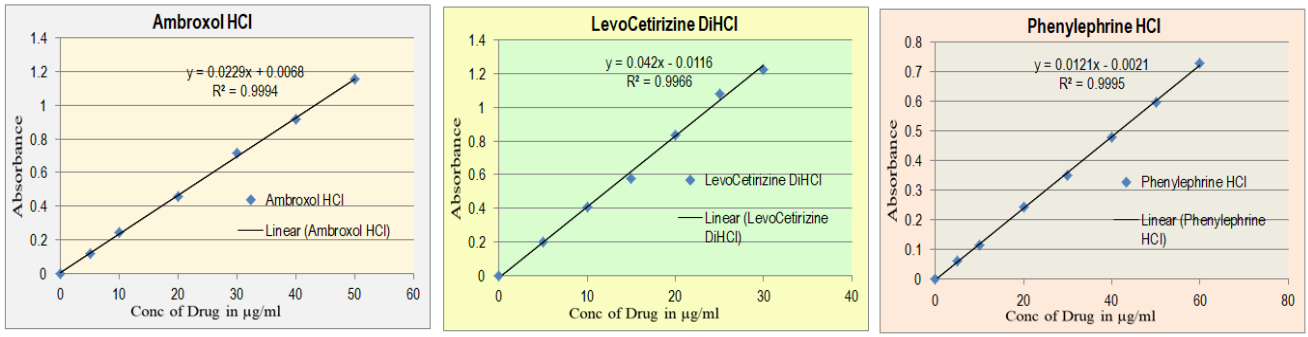


Figure 7: Calibration graph of ARX, LCZ and PER of linearity study

Table 3: Parameters of regression equation obtained in Microsoft excel office

Parameters	LCZ	ARX	PER
Detection wavelength	231 nm	244 nm	273 nm
Beer's law limit (µg/ml)	5 – 25 µg/ml	5 – 50 µg/ml	5 – 60 µg/ml
Correlation coefficient (r^2)	0.9966	0.9994	0.9995
Regression equation	y=0.042x-0.0116	y=0.0229x+0.0068	y=0.0121x-0.0021
LOD	0.086	1.051	1.025
LOQ	0.261	3.157	2.91

Assay of Formulation

The assay was carried out by calibration curve method. The spectra of formulation were obtained and calculated % of nominal conc and SD, data was found within acceptable limits are summarized in Table 4. The results indicated applicability of the method for estimation of Formulation.

Accuracy and Precision

The results of accuracy are summarised in Table No 5 a and b, the obtained results were within acceptable limit; and methods accuracy was justified by calculating % drug content. The precision study was carried out by performing assay of solutions; further the reproducibility in result was studied by interday and intraday precision. The values obtained SD and % RSD was shown methods precision and are summarised in Table 5 a and b.

Table 4: Results of assay of formulation by proposed method

Tablet sample	Drug Name	Label claim (mg/Tab)	% of Label claim estimated*	Amount Found in mg	Standard deviation	Relative Std Dev
Levocold	AMB	60	99.96	59.97	1.1384	1.1386
	LCZ	5	99.39	4.96	1.3959	1.4047
Levocet-D	PER	10	99.95	9.96	1.1384	1.1386
	LCZ	5	101.23	5.06	1.3959	1.3789

* Mean of six determinations

Table 5a: Results of accuracy and precision study of formulation ARX and LCZ

S. N.	Parameter	Level of study	Data Title	Amount%	S.D.	RSD
Form. of LCZ and ARX	Precision study of LCZ	Intraday Precision	Mean of Abs n= 6	103.342	1.9573	1.8941
	Precision study of ARX	Intraday Precision	Mean of Abs n= 6	102.306	0.7824	0.7648
	Accuracy study of LCZ	80%	% Recovery	102.245	1.8818	1.8464
		100%		99.775	3.8305	3.8792
		120%		101.478	1.9443	1.9157
	Accuracy study of ARX	80 %	% Recovery	101.740	1.5167	1.4907
		100 %		104.835	2.0125	1.9197
		120 %		104.228	3.6215	3.4746

Table 5b: Results of accuracy and precision study of formulation LCZ and PER

S. N.	Parameter	Level of study	Data Title	Amount %	S.D.	RSD
Form. of LCZ and PER	Precision study of LCZ	Intraday Precision	Mean of Abs n= 6	101.211	0.3647	0.3603
	Precision study of PER	Intraday Precision	Mean of Abs n= 6	104.553	0.3868	0.3699
	Accuracy study of LCZ	80%	% Recovery	100.956	1.1938	1.1824
		100%		101.715	2.2121	2.1748
		120%		103.098	1.9768	1.9174
	Accuracy study of PER	80 %	% Recovery	98.188	2.0016	2.0386
		100 %		98.608	0.7698	0.7798
		120 %		102.605	1.6321	1.5907

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of ARX, LCZ and PER by the proposed method were found within acceptable limit.

Robustness and Ruggedness

Robustness was studied and capacity of analytical procedure to measure analyte remained unaffected by small but deliberate variations in method parameter like variation in the wavelength ± 1 nm, variation in the solvent strength by ± 0.1 %. The analytical method was found rugged during development; similarity the result was produced by performing the analysis by different analyst.

CONCLUSION

The method was developed with eco-friendly and readily available distilled water solvent. Both the formulations were estimated by the proposed method and satisfactory results were obtained. The obtained results of the methods were within acceptable limits given in the guidelines and official books. The validated method is economical, precise, accurate, robust and reproducible hence can be routinely used for estimation of both these dosage forms.

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