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A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF OLMESARTAN MEDOXOMIL AND CILNIDIPINE IN ITS PHARMACEUTICAL DOSAGE FORM BY UPLC AS PER ICH GUIDE LINES

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ABSTRACT

A simple, accurate, precise, sensitive, rapid UPLC method has been developed and validated for determination of Olmesartan medoxomil and Cilnidipine in its pharmaceutical dosage form. Chromatographic separation was achieved on a BEH C18 column (100 ×2.1mm,1.7), by a mobile phase consisted of Ph3.5 buffer, maintained with Roth phosphoric acid and methanol in 35:65(V/V) ratio with a flow rate of 0.3 ml/min. The detection wavelength was set at 254 nm. Olmesartan medoxomil and Cilnidipine was subjected to different stress conditions. The degradation products, when any, were well resolved from the pure drug with significantly different retention time values. The method was linear (r = 0.999) at a concentration range of 0.2-0.3 µg/ml. The intra and inter day precisions were satisfactory the relative standard deviations did not exceed 2%. The accuracy of the method was proved the mean recovery of Olmesartan medoxomil and Cilnidipine was 99.04-101.58%**.** The proposed method has high throughput as the analysis involved short run-time (3.20 mines). The method met the ICH/FDA regulatory requirements. The proposed method was successfully applied for the determination of Olmesartan medoxomil and Cilnidipine with acceptable accuracy and precisions. The results demonstrated that the method can be applied successfully for routine use in quality control industry laboratories.

KEYWORDS

Olmesartan medoxomil and Cilnidipine, UPLC, BEH and ICH.

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INTRODUCTON

Cilinidipine is a dihydropyridine calcium channel blocker and chemically it is 3-O-(2- Methoxyethyl)5-O-[(E)-3-phenylprop-2-enyl]2,6-dimethyl-4-(3-nitro phenyl)-1,4 dihydropyridine-3,5-dicarboxylate and it is a unique Ca2+ channel blocker with an inhibitory action on the sympathetic N-type Ca2+ channels, which is used for patients with hypertension and its Molecular formula C27H28N2O7 Molecular weight :

492. Olmesartan is an angiotensin II receptor blocker and chemically it is 4-(2-hydroxypropan-2- yl)-2-propyl-1-({4-[2-(1H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-1H-imidazole-5- carboxylic acid. The molecular weight is 558.59, molecular formula is C29H30N6O6. It selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Literature review reveals very few methods are reported for the assay of Olmesartan medoxomil and Cilnidipine in Tablet dosage forms using RP-HPLC and UV method and no method has been developed by UPLC. The proposed UPLC method utilizes economical solvent system and having advantages like less time consuming, better retention time, less flow rate, very sharp and symmetrical peak shapes. The aim of the study was to develop a simple, precise, economic and accurate UPLC method for the estimation of Olmesartan medoxomil and Cilnidipine in Tablet dosage forms.

MATERIALS AND METHODS

UPLC system (Waters Acquity equipped with Empower 2 software, auto sampler and PDA detector), Waters Acquity UPLC C_{18} BEH column 2.1X50mm.

Chemicals and reagents

Gifted sample of Olmesartan medoxomil and Cilnidipine pure sample and dosage form “Benicar” marketed by REDDY’S was purchased from local pharmacy. Other chemicals all are of HPLC grade and LR grade.

Preparation of Potassium Phosphate buffer

Weighed 6.8grams of Potassium di hydrogen orthophosphate into 1000ml beaker dissolved and diluted to 1000ml with HPLC water. Adjust end the pH to 3.5 with Orthophosporic acids.

Preparation of mobile phase

Mix a mixture of above buffer 350 mL (35%) and 650 ml methanol HPLC (65%) and degas in ultrasonic water bath for 5 minutes. Filter through 4.5 µ filter under vacuum filtration.

Diluents Preparation

Use the Mobile phase as Diluents.

Preparation of the Olmesartan and Cilnidipine Standard and Sample Solution.

Standard Solution Preparation

Accurately weigh and transfer 20 mg of Olmesartan and 10 mg of Cilnidipine working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution

Further pipette 1.0 ml of Olmesartan and Cilnidipine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3 ml and 3ml of Olmesartan and Cilnidipine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation

Accurately weigh and transfer equivalent to 20 mg of Olmesartan and 10mg Cilnidipine equivalent weight of the sample into a 10ml clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution

Further pipette 1.0 ml of Olmesartan and Cilnidipine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3 ml and 3ml of Olmesartan and Cilnidipine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Olmesartan and Cilnidipine peaks and calculate the % Assay by using the formulae. Method optimization. The chromatographic separation was performed using Waters Acquity UPLC BEH C18 (100 mm X 2.1 mm, 1.7µm) column. For selection of mobile phase, various mobile phase compositions were observed for efficient elution and good resolution. The mobile phase consisting of Mobile phase [pH 3.5 Buffer: ACN (50:50 % v/v)] was found to be the optimum composition for efficient elution of analyte. The mobile phase was injected to the column at a flow rate

of 0.3 ml/min for 3min. The column temperature was maintained at 25^0C. The analyte was monitored at 254 nm using UV-detector. The retention time of the drugs was found to be 0.594min for OLM, 0.819 min for CIL. Mobile phase was used as diluent during the standard and test samples preparation. The optimized chromatographic conditions are mentioned in Table No.1 and chromatogram for standard was shown in the Figure No.3.

RESULTS

Method Validation

System suitability

System suitability tests are an integral part of method validation and are used to ensure adequate performance of the chromatographic system. Retention time (RT), number of theoretical plates (N) or column efficiency and tailing factor (T) were evaluated for six injections of standard solution at a solution of 5µg/ml of Olmesartan medoxomil and Cilnidipine. The results are tabulated in the Table No.2 and the chromatogram was shown in the Figure No.4.

Specificity

Specificity is the ability of analytical method to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample. The specificity of method was determined by spiking possible impurities at specific level to standard drug solution (5ppm). The diluent and placebo solutions were also injected to observe any interference with the drug peak. There was no blank and placebo interference was found.

Linearity

Linearity is the ability of the method to produce results that is directly proportional to the concentration of the analyte in samples with given range. The linearity of Olmesartan medoxomil and Cilnidipine was in the concentration range of 5-25ug/ml. From the linearity studies calibration curve was plotted and concentrations were subjected to least square regression analysis to calculate regression equation. The regression coefficient was found to be 0.9999 shows good linearity. The results are tabulated in the Table No.4 and the chromatogram was shown in the Figure No.7, 8, 9.

Accuracy

Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Accuracy of the method was evaluated by standard addition method. Recovery of the method was determined by spiking an amount of the pure drug (50%, 100%, 150%) at four different concentration levels in its solution has been added to the pre analyzed working standard solution of the drug. The results are tabulated in the Table No.5, 6, 7.

Precision

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The Precision expressed as standard deviation or relative standard deviation.

System precision

System precision was performed by injecting a standard solution of Olmesartan medoxomil and Cilnidipine for six times. The results are tabulated in the Table No.8.

Method precision

Method precision was performed by analyzing a sample solution of Olmesartan medoxomil and Cilnidipine by injecting six replicates of the same sample preparations at a concentration of 0.3ppm/mL. The results are tabulated in the Table No.9.

Intermediate precision (Ruggedness)

Intermediate precision was performed by analyzing a standard and sample solution of Olmesartan medoxomil by injecting six replicates of the same standard and sample preparations at a concentration of 0.3 ppm/mL. The results are tabulated in the Table No.9.

Robustness

Robustness shows the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. The results are tabulated in the Table No.4.

LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. The results shows, the limit of detection

with a signal to noise ratio of 3:1 was found to be 0.010 µg/ml. The limit of quantification with a signal to noise ratio of 10:1 was found to be 0.032 µg/ml.

DISCUSSION

System suitability

From the system suitability studies it was observed that retention time of Olmesartan medoxomil and cilnidipine was found to be 3.42 min. % RSD of peak area was found to be 0.4 for olm, 0.7 for cil. Theoretical plates were found to be more than 2568. USP tailing factor was found to be 1. All the parameters were within the limit.

Specificity

The Chromatograms of Standard and Sample are identical with nearly same Retention time. There is no interference with blank and placebo to the drugs. Hence the proposed method was found to be specific.

Linearity

From the Linearity data it was observed that the method was showing linearity in the concentration range of 5-25µg/ml. Correlation coefficient was found to be 0.9999. 4.4.

Accuracy

The recoveries of pure drug from the analyzed solution of formulation were in the range of 98%-102%, which shows that the method was accurate**.**

Precision

System precision

The percentage relative standard deviation (RSD) for the peak area 1.0.

Method precision

The percentage relative standard deviation for the assay values found to be 1.06

Ruggedness

Comparison of both the results obtained for two different Analysts shows that the method was rugged for Analyst-Analyst variability. The %RSD for intermediate precision was 1.0.

Robustness

As the % RSD of retention time and asymmetry were within limits for variation in flow rate (± 0.2 ml). Hence the allowable flow rate should be within 0.3 ml to 1.7 ml. As the % RSD of retention time and asymmetry were within limits for variation (+ 50 C) in column oven temperature. Hence the allowable variation in column oven temperature is + 50 C. The results obtained were satisfactory and are in good agreement as per the ICH guidelines.

Table No.1: Optimized Chromatogram Conditions Olmesartan and Cilnidipine

S.No	Equipment	Ultra Performance Liquid Chromatography Equipped with Auto Sampler and PDA Detector
1	Column	Inspire C18 (2.1 x 50mm, 1.8µm,) or equivalent
2	Flow rate	0.3 mL per min
3	Wavelength	254 nm
4	Injection volume	5 µl
5	Column oven	Ambient
6	Run time	4 min

Table No.2: System Suitability and Robustness Data for Olmesartan Medoxomil and Cilnidipine
System Suitability Results for Olmesartan

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.2	2658	1.41
2	0.25	2784.08	1.43
3	0.3	2754	1.42

System Suitability Results for Cilnidipine

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.2	2874	1.8
2	0.25	2927.52	1.51
3	0.3	3678	1.56

System Suitability Results for Olmesartan

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2568	1.54
2	*Actual	2784.08	1.43
3	10% more	2862	1.29

Table No.3: System Suitability Results Cilnidipine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3654	1.68
2	*Actual	2927.52	1.51
3	10% more	3921	1.53

Table No.4: The Accuracy Results for Olmesartan

S.No	%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
1	50%	726004	10	10.23	102.18%	101.0%
2	100%	1418064	20	19.98	99.89%	
3	150%	2149402	30	30.28	100.94%	

Table No.5: The Accuracy Results for Cilnidipine

S.No	%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
1	50%	966975	5	5.01	100.26%	99.36%
2	100%	1912156	10	9.91	99.13%	
3	150%	2855477	15	14.80	98.69%	

Table No.6: The Precision Results for Olmesartan

S.No	Injection	Area
1	Injection-1	1377208
2	Injection-2	1377278
3	Injection-3	1377914
4	Injection-4	1376060
5	Injection-5	1389304
6	**Average**	1379553
7	**Standard Deviation**	5491.9
8	**% RSD**	0.4

Table No.7: The Precision Results for Cilnidipine

S.No	Injection	Area
1	Injection-1	2043780
2	Injection-2	2025801
3	Injection-3	2022977
4	Injection-4	2033312
5	Injection-5	2057106
6	**Average**	2036595
7	**Standard Deviation**	14009.5
8	**% RSD**	0.7

Table No.8: Linearity Results for Olmesartan

S.No	Linearity Level	Concentration	Area
1	I	20ppm	676099
2	II	40 ppm	1226320
3	III	60 ppm	1705005
4	IV	80 ppm	2350334
5	V	100 ppm	2904688
Correlation Coefficient			0.999

Table No.9: Linearity Results for Cilnidipine

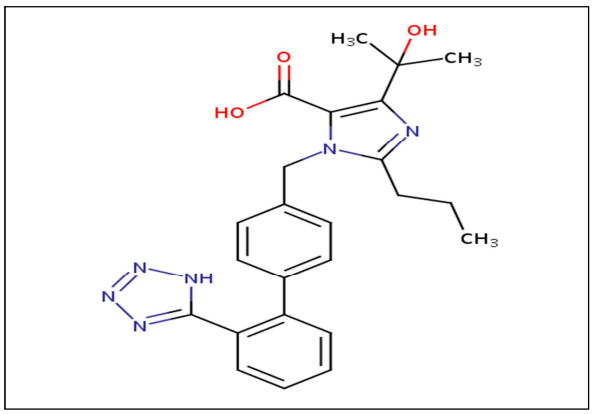
S.No	Linearity Level	Concentration	Area
1	I	30ppm	909469
2	II	60 ppm	1610151
3	III	90 ppm	2374209
4	IV	120 ppm	3164470
5	V	150 ppm	3837500
Correlation Coefficient			0.999

Table No.10: The Interday Precision Results for Olmesartan

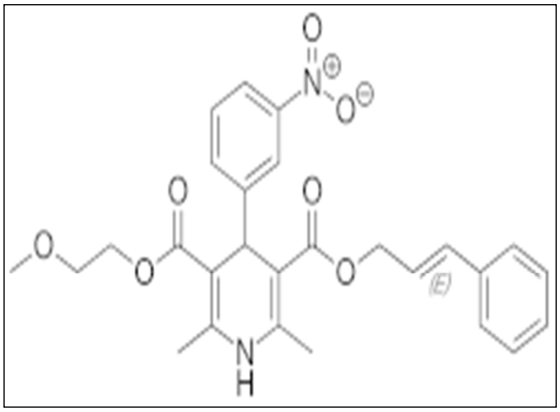
S.No	Injection	Area
1	Injection-1	1350592
2	Injection-2	1372286
3	Injection-3	1354685
4	Injection-4	1360384
5	Injection-5	1332603
6	Injection-6	1351712
7	**Average**	1353710.3
8	**Standard Deviation**	13036.7
9	**% RSD**	1.0

Table No.11: The Interday Precision results for Cilnidipine

S.No	Injection	Area
1	Injection-1	1984941
2	Injection-2	2023618
3	Injection-3	2002586
4	Injection-4	2011358
5	Injection-5	1970501
6	Injection-6	1985667
7	**Average**	1996445.2
8	**Standard Deviation**	19596.3
9	**% RSD**	1.0



(A) Olmesartan



(B) Cilnidipine

Figure No.1: The Chemical Structures of Olmesartan (A) and Cilinidipine (B)

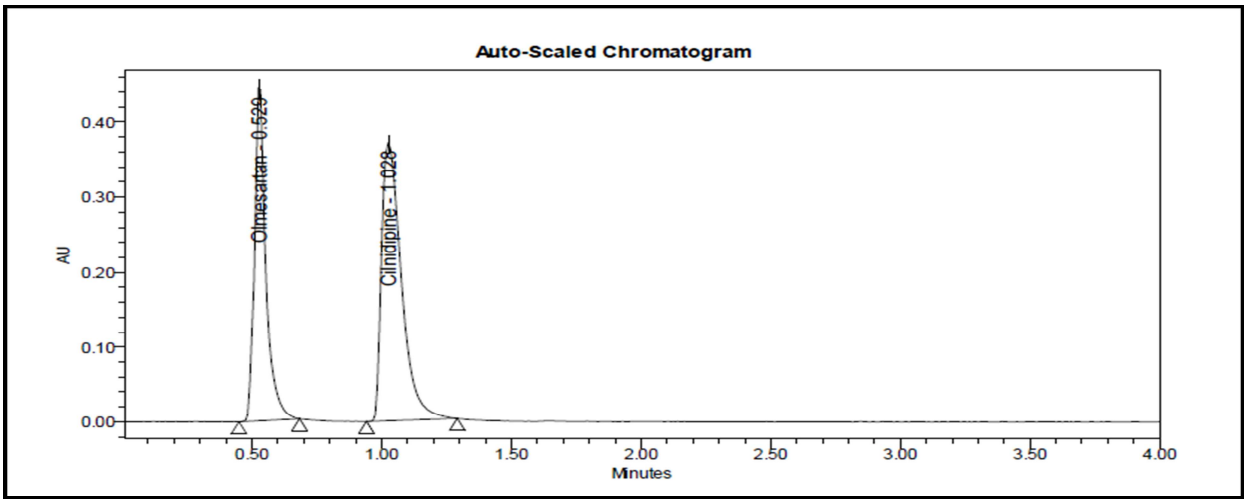


Figure No.2: Standard Chromatogram

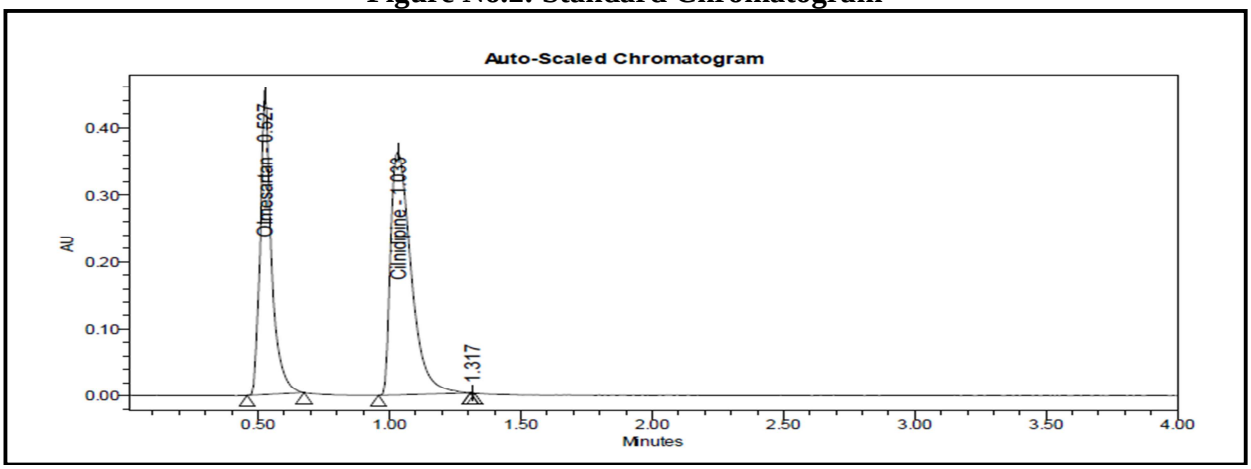


Figure No.3: Standard Chromatogram

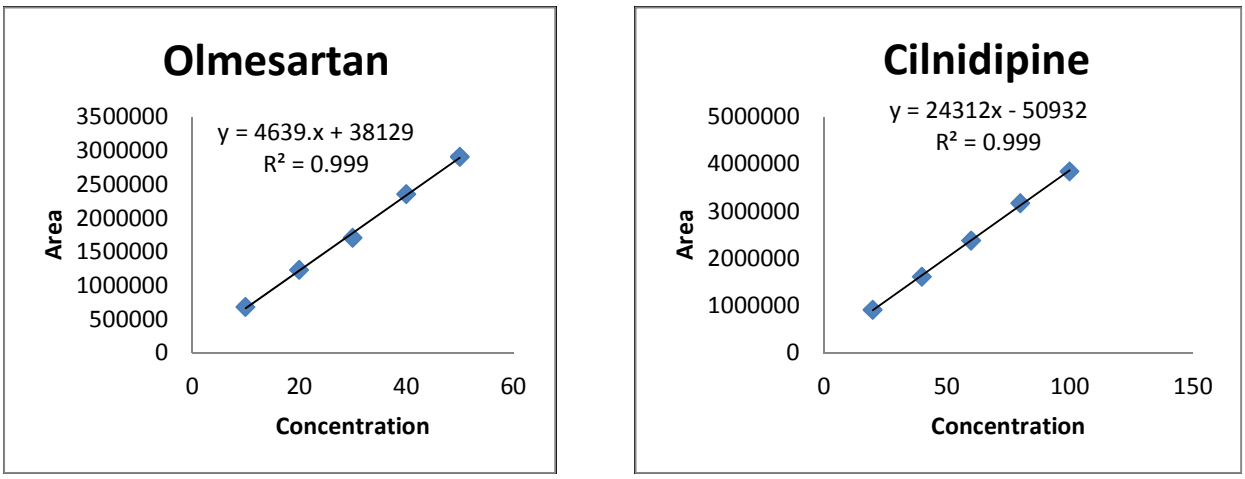


Figure No.4: Linearity Curve for OLN and CLE

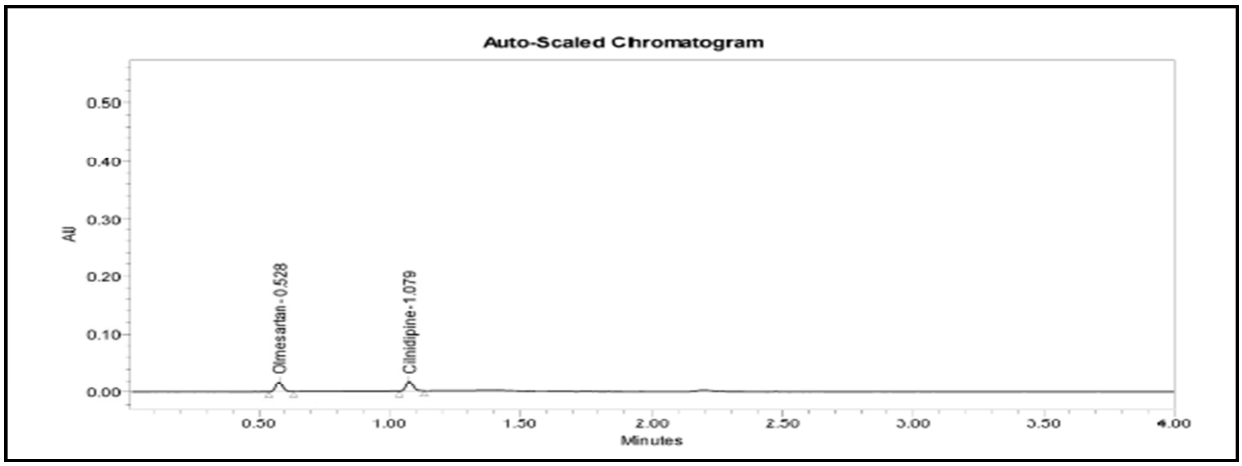


Figure No.5: Chromatogram for Specificity

CONCLUSION

Finally it concludes that all the parameters are within the limits and meet the acceptance criteria of ICH guidelines for method validation. The proposed method was simple, accurate, specific, precise, robust, rugged and economical. Hence this method is validated and can be used for routine sample analysis.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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