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Original Article

DEVELOPEMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND OLMESARTAN MEDOXOMIL IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

AMIT S. MINASE*, MANJUSHA N. DOLE, SANJAY D. SAWANT

Department of Pharmaceutical Chemistry, Smt. Kashibai Navale College of Pharmacy, Kondhwa, Pune-411048, Maharashtra, India
Email: amitminase@gmail.com

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ABSTRACT

An economical, precise, rapid and accurate RP HPLC method has been developed for the simultaneous estimation of Olmesartan Medoxomil and Cilnidipine in bulk and tablet dosage form. Separation was carried out on Jasco HPLC system equipped with HiQ sil C18 column (250 × 4.6 mm i.d.) and PDA detector using Methanol: 40 mM Potassium dihydrogen ortho phosphate buffer (90:10 v/v) as the mobile phase.Ortho-phosphoric acid was used to adjust pH to 3.0, and detection was carried out at 254 nm. Results were linear in the range of 5-30 µg/ml for Cilnidipine and 10-50 µg/ml for Olmesartan Medoxomil respectively. The method was successfully applied for the analysis of drugs in pharmaceutical formulation. Results of the analysis were validated statistically and by recovery studies.

Keywords: Cilnidipine, Olmesartan Medoxomil, RP-HPLC.

INTRODUCTION

Cilnidipine (CILNI) chemically, 1,4-Dihydro- 2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid 2-methoxyethyl(2E)-3-phenyl-propenyl ester is a dual blocker of L-type voltage-gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals[1]. Olmesartan Medoxomil (OLME) chemically, 2,3-dihydroxy-2-butenyl-(1-hydroxy-1-methyl ethyl)-2-propyl-1-[P-(O-1H-tetrazole-5-ylphenyl)benzyl] imidazole-5- carboxylate, cyclic 2,3-carbonate. Olmesartan Medoxomil is a prodrug, which after ingestion liberates the only active metabolite, Olmesartan. Olmesartan is a competitive and selective angiotensin 2 receptor antagonist. Both drugs used in combination to treat hypertension [1]. Literature survey reveals that cilnidipine can be estimated by spectrophotometric [2, 3, 4], reverse phase high-performance liquid chromatography (RP-HPLC) [5, 6] and high performance thin layer chromatography (HPTLC) [7.8] methods either as a single or in combination with other drugs in pharmaceutical preparations. Analytical methods reported for OLME includes spectrophotometric [9, 10], HPLC [11, 12, 13], and HPTLC [14, 15] either as a single drug or in combination with other drugs. Literature survey reveals that not a single HPLC method of analysis has yet been reported for simultaneous analysis of CILNI and OLME. The objective of the present investigations was to develop a rapid, accurate, economical and validated reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous estimation so that can play important role in quantification of CILNI and OLME in bulk and tablet dosage form The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [16].

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical grade working standards of CILNI were obtained as gift sample from J. B. Chemicals and Pharmaceuticals Ltd. (Daman) and OLME from Macleods Pharmaceutical Ltd. (Mumbai) used as such without further purification. The pharmaceutical dosage form used in this study was Nexvas-o tablets (Macleods Pharmaceutical Ltd., Mumbai, India), labeled to contain 10 mg of CILNI and 20 mg of OLME procured from the local market. Methanol (HPLC grade), Potassium dihydrogen orthophosphate (AR grade), Ortho phosphoric acid (AR grade) purchased from Thomas Baker (chemical) Pvt. Ltd. (Mumbai) and double distilled water was used for analysis.

Instrumentation and Chromatographic conditions

Jasco HPLC system consisting of Jasco PU-2089 plus HPLC pump with Jasco MD-2018 plus PDA detector and ChromNAV software was used for analysis. Separation was carried out on HiQ sil C18 (250 x 4.6 mm i.d.) column using mobile phase methanol: 40mM potassium dihydrogen orthophosphate buffer (90:10 v/v) and pH adjusted to 3.0 by orthophosphoric acid at flow rate of 1 ml/ min. 20 µl Samples were injected using syringe and detection was carried out at 254 nm.

Preparation of standard stock solutions

Standard stock solution of CILNI and OLME was prepared separately by dissolving 10 mg of drug in 10 ml methanol to get concentration of 1000 µg /ml from which 1 ml of solution was further diluted to 10 ml with mobile phase to get a working standard solution having concentration 100 µg /ml for both the drugs.

Procedure for analysis of tablet formulation

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10 mg of CILNI and 20mg of OLME was weighed and transferred to100 ml volumetric flask containing about 80 ml of methanol and sonicated for 20 min and volume was made up to the mark with the methanol.

The solution was filtered through Whatman paper No. 41. Further dilution are done with the mobile phase to get solution of concentration 10µg/ ml for OLME and 5 µg/ ml for CILNI After setting the chromatographic conditions and stabilizing the instrument, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

System Suitability

The system suitability was assessed by six replicate injections of the mixture containing 5µg/ ml and 10 µg/ ml of CILNI and OLME respectively. The resolution, peak asymmetry, number of theoretical plates, and tailing factor were calculated as represented in Table 2.

Method Validation

The method was validated for linearity, accuracy and intra-day and inter-day precision and robustness, in accordance with ICH guidelines. [16]

Linearity

The stock solution of OLME and CILNI was prepared by dissolving 10mg of both drugs in 10 ml methanol separately to obtain a concentration of 1000µg/ml. From this stock solution further dilutions were made in mobile phase to obtain concentrations of 5-30µg/ml and 10-50µg/ml for CILNI and OLME respectively.

A graph was plotted as concentration of drugs versus peak area response it was found to be linear for both the analytes. (Shown in Figure 2) From the standard stock solution, a mixed standard solution was prepared containing 10µg/ml of OLME and 5µg/ml of CILNI. The system suitability test was performed on six replicate injections of mixed standard solution.

Table 1: It shows analysis data of nexvas-o tablet formulation with statistical evaluation

Amt of Drug Present (mg)		Amt of Drug Found (mg)		%Amount estimated*		%RSD	
CILNI	OLME	CILNI	OLME	CILNI	OLME	CILNI	OLME
10	20	9.96	20.22	99.89	101.15	0.3040	1.53

*Average of six determinations

Table 2: It shows system suitability parameters for RP-HPLC method

Parameter	CILNI	OLME
Retention time (min.)	6.32	2.47
Theoretical Plates	4723	2823
Resolution	12.23	
Tailing factor	1.66	0.90

The obtained values describe the suitability of the system for the analysis of these drugs in combination. Mean retention time was found to be 2.47 for OLME and 6.32 for CILNI respectively. The representative chromatogram of the standard solution of mixture is shown in Figure 1.

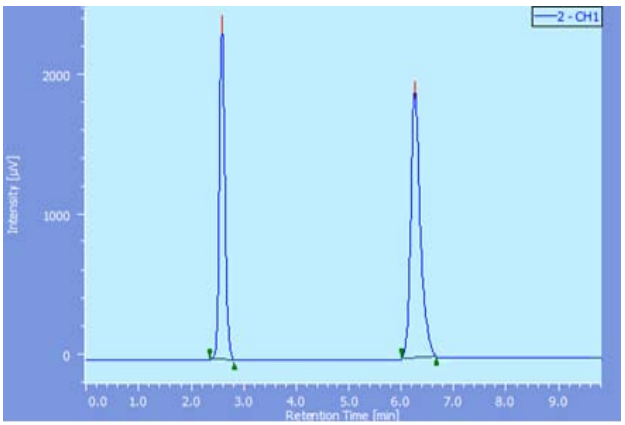

Fig. 1: It shows representative chromatogram obtained for standard mixture of OLME (10 µg/ml, 2.47 min) and CILNI (5 µg/ml, 6.32 min)

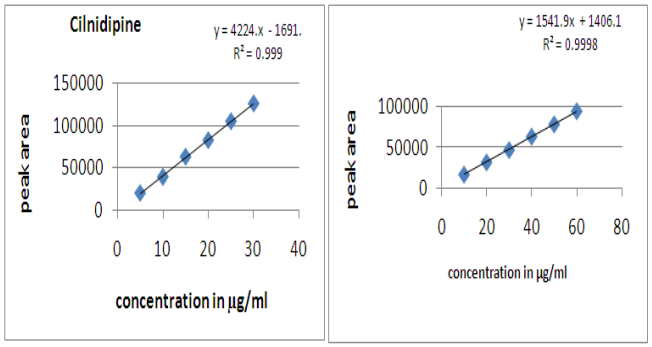

Fig. 3: It shows calibration curve for CILNI and OLME

Precision

One set of three different concentrations of mixed standard solutions of OLME and CILNI were prepared. All the solutions were analyzed thrice, in order to record interday and intraday variations in the results. For Inter day variations study three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days. The peak areas were recorded and relative standard deviation (RSD) was calculated for both series of analysis.

Table 3: It shows precision studies for CILNI and OLME

Drug	Drug content %	Std. Dev.	%RSD*	SE
Inter-Day Precision				
CILNI	99.70	0.408	0.409	0.16
OLME	99.66	0.334	0.335	0.13
Intra-Day Precision				
CILNI	98.11	0.809	0.825	0.33
OLME	99.06	0.214	0.216	0.028

* Average of three determinations; RSD relative standard deviation**:** SE standard error

Accuracy

To check the accuracy of the method recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80%, 100% and 120%. The percentages of recoveries were calculated,

the results of which are represented in Table 4.

Table 4: Recovery studies of OLME and CILNI

DRUG	Amount of drug taken* (µg/ml)	Amount of drug added* (µg/ml)	Amount of drug found* (µg/ml)	%amount of drug recoverd *	%RSD
CILNI	10	08	17.9092	99.49	0.3993
	10	10	19.7133	98.56	0.7331
	10	12	21.7642	98.92	0.8523
OLME	20	16	36.1499	100.41	0.7422
	20	20	39.8944	99.73	0.6259
	20	24	44.0034	100.00	0.4693

* Average of three determinations; RSD relative standard deviation

Limit of detection and Limit of quantitation

Limit of detection and Limit of quantitation were calculated as 3.3 σ /S and 10 σ /S respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following three factors were selected for change: flow rate of the mobile phase (1 ± 0.1 ml /min), pH of mobile phase (3 ± 0.1) and concentration of buffer (10 ± 1ml). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

Results were found to be linear in the concentration range of 10-50 µg /ml for OLME and 5-30 µg /ml for CILNI with r^2 = 0.999 respectively. The proposed method was also evaluated by the assay of commercially available tablets containing OLME and CILNI. The % assay was found to be 99.79 % for CILNI and 101.15% for OLME.The % recovery was found to be in the range of 98.56 to 99.49 for CILNI and 99.73 to 100.41 for OLME. The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. Robustness of the method checked after deliberate alterations of the analytical parameters shown no marked changes in the chromatograms (RSD NMT 2%), which demonstrated that the RP-HPLC method developed is robust. The summary of validation parameters of proposed HPLC method is given in Table 5.

Table 5: It shows summary of validation parameters of proposed RP-HPLC method

Parameter	CILNI	OLME
Linearity range (ng/ spot)	100-200	200-400
Correlation co-efficient	0.995	0.996
Slope (m)	13.41	4.140
Intercept (c)	726.0	584.8
Precision (intraday) %RSD	1.44	1.28
Precision (interday) %RSD	1.71	1.59
Accuracy	98.61 to 100.87	98.48 to 99.51
LOD (µg /ml)	2.00	2.24
LOQ (µg /ml)	6.009	6.69

CONCLUSION

The developed and validated RP-HPLC method is found to be rapid, accurate, precise and robust, thus can be used for routine analysis of olmesartan medoxomil and cilnidipine in combined tablet dosage form.

CONFLICT OF INTERESTS

Declared None

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