

# A Sensitive RP-HPLC Method for Simultaneous Estimation of Diethylcarbamazine and Levocetirizine in Tablet Formulation

J. MAHESH REDDY, M. R. JEYAPRAKASH\*, K. MADHURI, S. N. MEYYANATHAN AND K. ELANGO

Department of Pharmaceutical Analysis, J. S. S College of Pharmacy, (Off Campus College of JSS University, Mysore), Rock Lands, Ootacamund-643 001, India

Reddy, *et al.*: RP-HPLC Method for Estimation of Diethylcarbamazine and Levocetirizine

A simple, sensitive and reproducible method was developed and validated for the simultaneous estimation of diethylcarbamazine and levocetirizine in its tablet formulation by reverse phase high performance liquid chromatography using Waters1515 HPLC with UV detector at the  $\lambda_{\max}$  of 224 nm, using Princeton Sphere-100 C<sub>18</sub> (250×4.6 mm, 5  $\mu$ ) column. The mobile phase used was 20mM potassium dihydrogen orthophosphate buffer (pH: 3.2):acetonitrile (50:50 v/v) with isocratic flow (flow rate 1 ml/min) and the pH was adjusted with orthophosphoric acid. Losartan potassium was used as an internal standard. The compounds diethylcarbamazine, levocetirizine and losartan potassium were eluted at 2.12, 4.27 and 5.96 min, respectively. The peaks were eluted with better resolution. The method was accurate with assay values of 96.32 and 93.04% w/w, precise (%RSD) with intra-day 1.72 and 1.89 and inter-day 1.85 and 1.92, recoveries 102.86 and 101.1% w/w, which are very sensitive with limit of detections (LOD)'s 75, 50 ng/ml and limit of quantification (LOQ)'s 100, 75 ng/ml and linear with R<sup>2</sup> values 0.994 in the range of 5 to 30  $\mu$ g/ml 0.1 to 1  $\mu$ g/ml for diethylcarbamazine and levocetirizine, respectively. Hence this method can be applied for quantification of different formulations containing diethylcarbamazine and levocetirizine simultaneously.

**Key words:** C<sub>18</sub> column, diethylcarbamazine, levocetirizine, RP-HPLC, validation

Diethylcarbamazine (DEC) is a piperazine anthelmintic agent indicated for the treatment of individual patients with lymphatic filariasis, tropical pulmonary eosinophilia and loiasis. The chemical name of the drug is N,N-diethyl-4-methylpiperazine-1-carboxamide citrate<sup>[1]</sup>. It acts by inhibiting arachidonic acid metabolism and it is a polar compound.

Levocetirizine (LEVC) is a third generation non-sedative antihistamine developed from second-generation antihistamine; cetirizine. Chemically LEVC is active enantiomer of cetirizine. The chemical name is 2-(2-(4-((R)-(4-chlorophenyl)-phenyl-methyl) piperazin-1-yl) ethoxy) acetic acid dihydrochloride. It is more effective with fewer side effects than second generation drugs. It works by blocking histamine receptors<sup>[2]</sup> and it is polar compound in nature.

Both drugs have good pharmacological actions. Many formulations are marketed individually or combination with other drugs. UV/Vis spectrophotometric methods<sup>[3-5]</sup>, high performance liquid chromatographic (HPLC) methods<sup>[6-9]</sup>, liquid chromatography-tandem mass spectroscopic (LC-

MS/MS) method<sup>[10]</sup> and gas chromatographic (GC) method<sup>[11]</sup> are available for estimation of DEC and LEVC in formulations individually or in combination with other compounds or in plasma samples. DEC and LEVC combined formulation is recently available marketed product. Literature survey showed that no HPLC method is available for estimation of these drugs simultaneously. The present study aims in developing RP-HPLC method for simultaneous estimation of these compounds in formulations.

All the chemicals used were of HPLC grade. Potassium dihydrogen orthophosphate was obtained from Qualigens fine chemicals, orthophosphoric acid and acetonitrile were obtained from Rankem Fine Chemicals, Mumbai, India. All the drugs DEC citrate, LEVC dihydrochloride and losartan potassium (IS) were purchased from Sigma Aldrich chemicals, Bangalore, India. Tablets of Levodec (equivalent to 150 mg of DEC and 2.5 mg of LEVC, Reign (India) Formulations Pvt. Ltd, Mettupalayam, India) were purchased from a local pharmacy. The ultra pure water used was collected from Millipore system.

The method development was performed with Waters 1515 HPLC system (Dual  $\lambda_{\max}$  2487 UV detector), Rheodyne 7725i injector with 20- $\mu$ l loop and the

\*Address for correspondence

E-mail: jpvis7@gmail.com

output signal was monitored and integrated using Breeze software (3.30 version), Shimadzu UV 1700 spectrophotometer for optimizing the wave length. Sartorius digital balance, Systronics pH meter and ultra sonicator were used. Shimadzu Prominence HPLC (LC-20AT pump, SPD-20A detector) was used for determining the ruggedness of the method. Mobile phase used was 20 M potassium dihydrogen orthophosphate buffer adjusted to pH 3.2 and acetonitrile with 50:50% v/v, it was filtered and degassed by ultra sonication.

Standard solutions of DEC, LEVC and IS were prepared separately at a concentration of 1 mg/ml and further dilutions were made to prepare working standard solutions used for validation studies. IS was maintained in each standard solution at a concentration of 50 µg/ml prepared from 1 mg/ml. All the dilutions were made with mobile phase.

Twenty tablets were powdered and average weight equivalent to one tablet was weighed and dissolved by adding mobile phase and sonicated for 30 min. It was filtered to remove the matrix by using Whatman filter paper of pore size 1 µ and made up the volume to 100 ml with mobile phase (solution A). From solution A, 1 ml was taken and made up the volume to 10 ml with mobile phase (solution B). From solution B, 1 ml was taken and 0.5 ml of 1 mg/ml of IS stock solution were mixed and made up the volume to 10 ml with mobile phase.

Separation of compounds was carried out by using reverse phase columns (Princeton SPHERE 100 C18, 250×4.6 mm id, 5µ, Hibar® C18 250×4.6 mm id, 5µ) and Princeton SPHER-100 C<sub>18</sub> (250×4.6mm 5 µ), was selected as the stationary phase, with a flow rate maintained at 1 ml/min with isocratic solvent pumping system. The analysis was done at ambient temperature (~20°). 20 µl of sample was injected and checked at wavelength of 224 nm.

The primary target in developing this method is to achieve simultaneous determination of DEC and LEVC in the tablet formulation under common conditions that will be applicable for routine quality control of the product in laboratories. Various mobile phases such as 20 mM potassium dihydrogen orthophosphate, Acetonitrile of pH 3.0 with 60:40 and 50:50 ratios were used as mobile phase. At 60:40 ratios, the peaks were eluted at 2.15 and 4.04 min with symmetry. At 50:50 ratios, the peaks were

eluted at 2.11 and 4.01 min with symmetric and well retained Peaks. For the present study 50:50 ratio was selected. Effect of flow rate Flow rates of 0.8 and 1.0 ml/min were used and chromatograms were recorded. When 0.8 ml/min was used, elution time of the peaks was 2.14 and 4.04 min, whereas 2.12 and 4.01 min at 1.0 ml/min. All these flow rates gave symmetric and well retained peak. For the present study the flow rate 1.0 ml/min was selected. Finally the present mobile phase with flow rate of 1ml/min was used for method development. The ionization of drugs takes place at pH 3.2. The UV wavelength was optimized at 224 nm for both detection and quantification. Losartan was selected as an internal standard. At this wavelength both drugs and internal standard gave significant absorption. No significant peaks were observed from the formulation matrix, indicating no interference from the matrix of the formulation. By this method the peaks were better resolved with retention times 2.11±0.05 min for DEC, 4.02±0.02 min for LEVC and 5.85±0.02 min for IS. The chromatogram of standard drugs with concentration of 20 µg/ml is shown in fig. 1.

The present method was validated as per ICH guidelines<sup>[12]</sup>. The peak purity of DEC, LEVC and IS were assessed by comparing the retention times (R<sub>t</sub>) of standard DEC, LEVC and IS. Good correlation was also found between the retention times of standards and sample of DEC, LEVC and IS.

Linearity of the responses of the two drugs were verified at six different concentration levels ranging from 5 to 30 µg/ml for DEC and 0.1 to 1 µg/ml for LEVC, respectively and 50 µg/ml of IS was maintained at each level. The calibration curve was constructed by plotting response factor (F) against concentration (C) of each drug. The regression equations obtained for two drugs were  $F=0.0038C+0.0003$  ( $R^2=0.9949$ ,  $n=6$ ) for DEC and  $F=0.0118C-0.0002$  ( $R^2=0.9979$ ,  $n=6$ ) for LEVC.

The developed method was applied in the estimation (assay) of DEC and LEVC in tablets. Two batches of the tablets were assayed and results are shown in Table 1, indicating that the amount of each drug in tablet samples met with requirements (92.5 to 107.5% of label claim for DEC and 90 to 110% of label claim for LEVC, respectively)<sup>[13]</sup>.

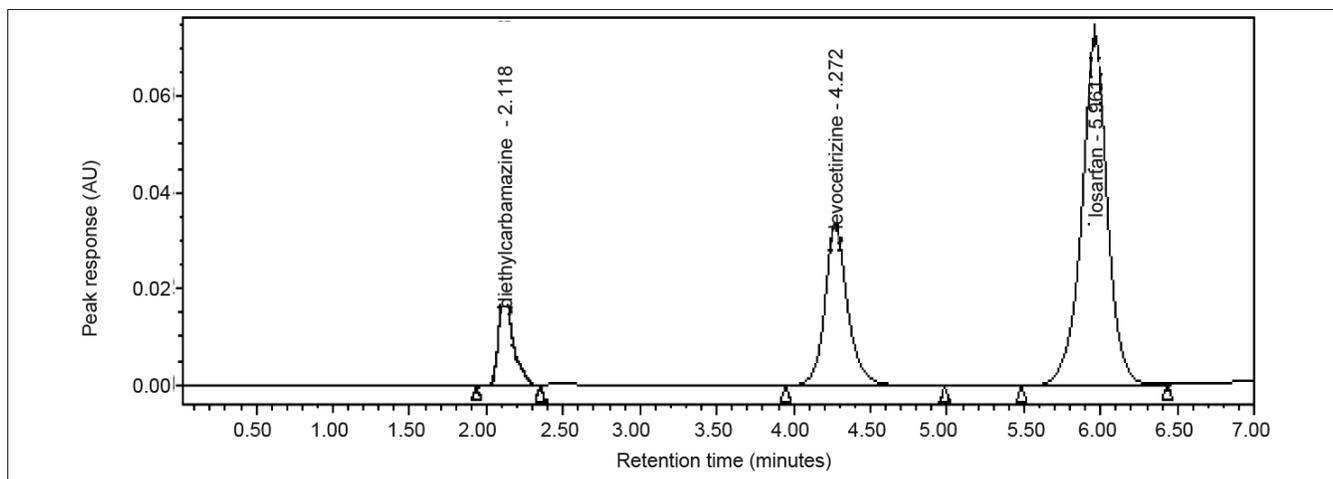


Fig. 1: Chromatogram of DEC, LEVC and IS  
Chromatograms of standard solutions of DEC (diethylcarbamazine, 20 µg/ml), LEVC (levocetirizine, 20 µg/ml) and IS (internal standard losartan, 20 µg/ml)

TABLE 1: ASSAY RESULTS FOR DEC AND LEVC

Drug name	Batch I (% Label claim ±SD*)	Batch II (% Label claim ±SD*)
DEC	95.1±1.72	97.53±1.53
LEVC	92.5±1.89	93.56±1.30

DEC is diethylcarbamazine, 150 mg per tablet and LEVC is levocetirizine, 2.5 mg per tablet. \*SD= Standard deviation (n=3)

Accuracy of the method was determined in terms of recovery by spiking to the pre-analyzed sample of two different concentrations 50 µg of DEC and 7.5 µg of LEVC standard drugs and the mixtures were reanalyzed by this method for three times. The mean recovery data was 51.43±0.52, 50.03±0.03 and percentage recovery was 102.86%, 101.10% for DEC and LEVC, respectively.

Precision study was performed to find out intra-day and inter-day variations in the estimation of DEC and LEVC of different concentrations, with the proposed method. The values of intra-day were 1.72, 1.85 and inter-day were 1.89, 1.92 for DEC and LEVC respectively. Percentage relative standard deviation (%RSD) of all the parameters is less than 2%, which indicates that the proposed method is precise.

The LOQ's by this method were found to be as 0.1 µg/ml and 0.075 µg/ml for DEC and LEVC respectively. Each value was verified by six individual injections of respective drug. The LOD's were found to be as 0.075 and 0.050 µg/ml for DEC and LEVC, respectively. These were confirmed by injecting respective standard drug solution at respective concentration for six times.

Ruggedness was determined on different HPLC systems such as Waters 2487 dual wavelength absorbance detector with a rheodyne 7725i, 20 µl loop using breeze as data station having 1515 solvent delivery system and a Shimadzu gradient system SPD M-10AVP photo diode array (PDA) detector with rheodyne 7725i, 20µl loop possessing LC-10 AT VP solvent delivery system using a Class-VP data station with different operators and different stationary phases § Princeton SPHERE 100 C18 (250×4.6 mm i.d., 5µ), § Hibar® C18 (250×4.6 mm i.d., 5µ) were used and the chromatograms were recorded. Robustness of the method was determined by making slight changes in the chromatographic conditions for change in the ratios of mobile phases, pH and flow rate as mentioned above. No marked changes were observed. System suitability parameters were checked which include theoretical plate/meter (5408 for DEC, 7641 for IS and 9412 for LEVC), resolution factor (1261 for DEC and 3.72 for LEVC), peak asymmetry factor (1.3 for DEC, 1.1 for IS and 1.1 for LEVC).

The results showed that the method provided adequate accuracy, precision, sensitivity, reproducibility with better resolution for the analysis of diethylcarbamazine citrate and levocetirizine dihydrochloride in formulations either simultaneously or individually. Thus it can be concluded that the proposed method can be used for the routine analysis of these two drugs in bulk as well as pharmaceutical preparations without any interferences.

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