IJPSR (2012), Vol. 3, Issue 09 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 June, 2012; received in revised form 06 July, 2012; accepted 27 August, 2012

A SENSITIVE RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DIETHYLCARBAMAZINE AND LEVO-CETIRIZINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Keywords:
C_{18} column,
Diethylcarbamazine (DEC),
Levocetirizine (LEVC),
RP-HPLC, validation

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ABSTRACT

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 Diethylcarbamazine and Levocetirizine simultaneously. HPLC-PDA detector at the λ_{max} of 253nm, using Hypersil-BDS C18 (250×4.6 mm. 5 µ) column. The mobile phase used was potassium dihydrogen orthophosphate buffer (pH: 5): acetonitrile (20:80 v/v) with isocratic flow (flow rate 1 ml/min) and the pH was adjusted with orthophosphoric acid. The compounds Diethylcarbamazine, Levocetirizine were eluted at 2.04, 5.54 min, respectively. The peaks were eluted with better resolution. The method was accurate with assay values of 99.67% and 99.81% w/w, precise (%RSD) with 0.3and 0.5, percentage recovery values of pure drug were in between 99.4% to 100% and 99% to 99.4% which are very sensitive with limit of detections (LOD)'s 2.42 and 0.08ppm and limit of quantification (LOQ)'s 7.42 and 0.2ppm, these results are within the range of limits 98% to 101% which indicates that the method was accurate and linear with R^2 values 0.999 in the range of 20to 120 µg/ml 0.8 to 4.8 µg/ml for Diethylcarbamazine and Levocetirizine, respectively.

INTRODUCTION: 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]. 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 and it is polar compound in nature. Both drugs have good pharmacological actions. Many formulations are marketed individually or combination with other drugs.

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UV/Vis spectrophotometric methods, High Performance Liquid Chromatographic (HPLC) methods, liquid chromatography-tandem mass spectroscopic (LC-MS/ MS) method and gas chromatographic (GC) method 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.

EXPERIMENTAL

Materials: 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, and Mettupalayam, India) were purchased from a local pharmacy. The ultra pure water used was collected from Millipore system.

1. **Procedure:** The method development was performed with Waters 1515 HPLC system (Dual λ_{max} 2487 UV detector), Rheodyne 7725i injector with 20-µl loop and the output signal was monitored and integrated using Breeze software (3.30 version), Shimadzu UV 1700 spectrophoto-meter for optimizing the wave length. Sartorious 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 potassium dihydrogen orthophosphate buffer adjusted to pH 5 and acetonitrile with 20:80% v/v, it was filtered and degassed by ultra sonication.

Preparation of Standard: Standard solutions of DEC, LEVC were prepared separately at a concentration of 1 mg/ml and further dilutions were made to prepare working standard solutions used for validation studies. All the dilutions were made with mobile phase.

Preparation of Sample: 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 Whatmann 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).

1. **Procedure:** Separation of compounds was carried out by using reverse phase columns using Hypersil-BDS C18 (250×4.6 mm. 5 µ) column 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 253 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 potassium dihydrogen orthophosphate, Acetonitrile of pH 5.0 with 20:80 ratios were used as mobile phase. At 20:80 ratios, the peaks were eluted at 2.047 and 5.540 min with symmetric and well retained Peaks. For the present study 20:80 ratio was selected.

   Effect of flow rate Flow rates of 0.9 and 1.1 ml/min were used and chromatograms were recorded. 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 5. The UV wavelength was optimized at 253 nm for both detection and quantification. At this wavelength both drugs 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 time's 2.047±0.05 min for DEC, 5.540±0.02 min for LEVC.

The present method was validated as per ICH guidelines. The peak purity of DEC, LEVC were assessed by comparing the retention times (R_T) of standard DEC, LEVC and IS. Good correlation was also found between the retention times of standards and sample of DEC, LEVC.

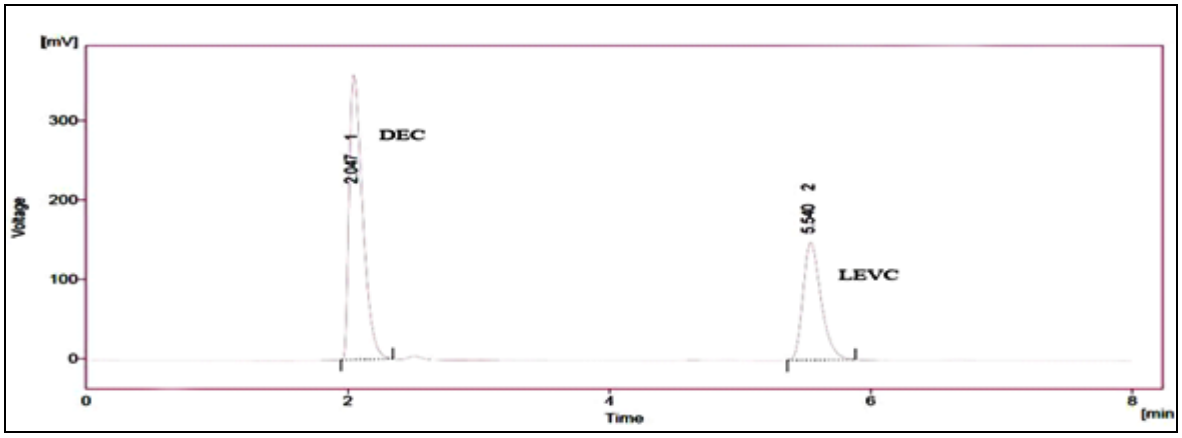


FIG 1: STANDARD CHROMATOGRAM OF DEC AND LEVC

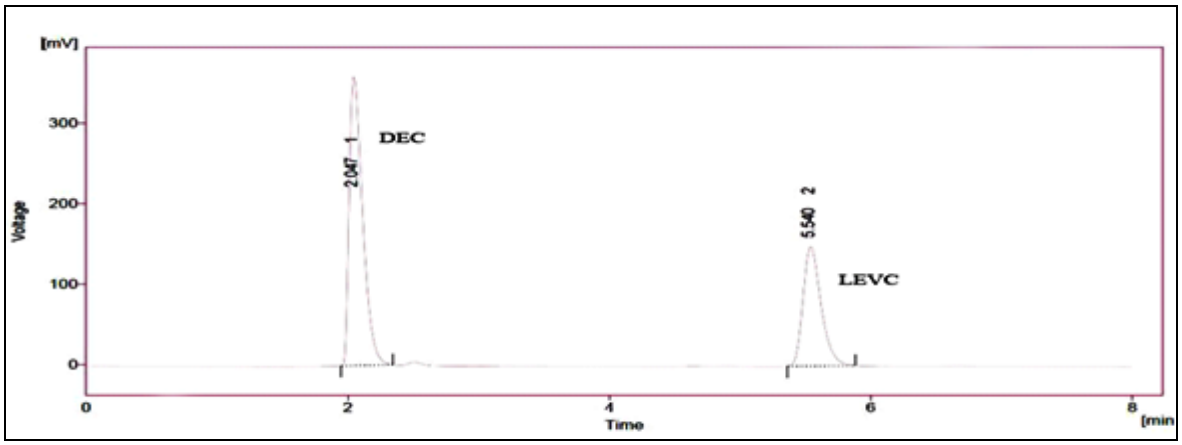
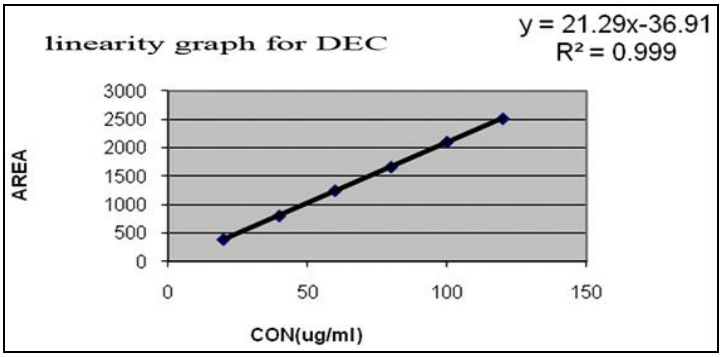


FIG 2: SAMPLE CHROMATOGRAM OF DEC AND LEVC

Linearity of the responses of the two drugs were verified at six different concentration levels ranging from 20to120 µg/ml for DEC and 0.8 to 4.8 µg/ml for LEVC, respectively. The calibration curve was constructed by plotting response factor (F) against concentration (C) of each drug.

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, indicating that the amount of each drug in tablet samples met with requirements (90 to110% of label claim for DEC and 90 to 110% of label claim for LEVC, respectively.

Accuracy: Accuracy of the method was determined in terms of recovery by spiking to the pre-analyzed sample of two different concentrations 100 µg of DEC and 4.0 µg of LEVC standard drugs and the mixtures were reanalyzed by this method for three times.



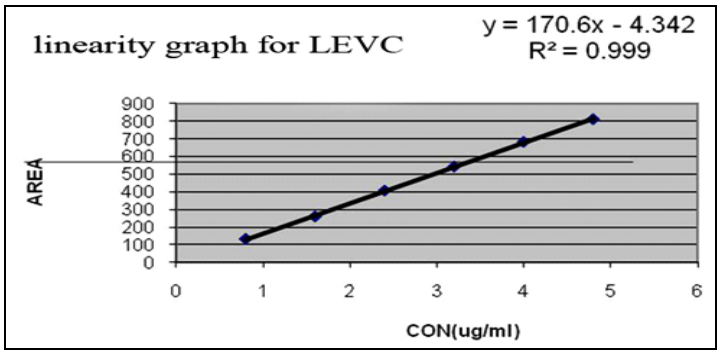


FIG 3: LINEARITY GRAPHS FOR DEC AND LEVC

Accuracy of DEC:

TABLE 1: ACCURACY RESULTS OF DEC

Sample Performed	Mean Sample peak area	Mean Standard peak area	Amount taken (µg/ml)	Amount found (µg/ml)	% Recovery
(80+10)	1896.833	2105.969	90	89.5645	99.51611
(100+10)	2310.812		110	109.9256	99.92323
(120+10)	2801.135		130	129.3773	99.52103

Accuracy of LEVC:

TABLE 2: ACCURACY STUDIES OF LEVC

Sample Performed	Mean Sample peak area	Mean Standard peak area	Amount taken (µg/ml)	Amount found (µg/ml)	% Recovery
(3.2+0.4)	608.118	682.7173	3.6	3.569183	99.14398
(4.0+0.4)	754.921		4.4	4.370849	99.33749
(4.8+0.4)	869.345		5.2	5.160354	99.23758

Precision: Precision study was performed to find out system and method precision variations in the estimation of DEC and LEVC of different concentrations, with the proposed method it was found that % RSD is not more than 1% (%RSD of DEC and LEVC are 0.3 and 0.5 respectively in system precision and 0.47 and 0.82 respectively in method precision); as these results are within the range of limits, which indicates that the proposed method has good reproducibility. The results are good for both method precession and system precision.

System Precision of DEC and LEVC:

TABLE 3: SYSTEM PRECISION STUDIES OF DEC AND LEVC

	Injection	Peak Areas of DEC	Cal. Amount (µg/ml) of DEC	Peak Areas of LEVC	Cal. Amount (µg/ml) of LEVC
Concentration 100%	1	2104.820	100.26	683.129	4.02
	2	2111.902	100.58	676.891	3.98
	3	2102.853	100.16	680.579	4.01
	4	2089.405	99.85	682.247	4.02
	5	2090.124	99.90	681.987	4.01
Statistical Analysis	**Mean**	2099.821	100.15	680.967	4.008
	SD		0.29		0.02
	% RSD		0.29		0.5

Method Precision of DEC and LEVC:

TABLE 4: METHOD PRECISION RESULTS OF DEC AND LEVC

	Injection	Peak Areas of DEC	Cal. Amount (µg/ml) of DEC	Peak Areas of LEVC	Cal. Amount (µg/ml) of LEVC
Concentration 100%	1	2099.921	100.02	678.246	3.99
	2	2103.007	100.21	687.274	4.05
	3	2072.828	99.07	683.923	4.02
	4	2102.756	100.16	688.614	4.06
	5	2086.005	99.71	675.818	3.98
Statistical Analysis	**Mean**	2092.903	99.834	682.775	4.02
	SD		0.47		0.035
	% RSD		0.47		0.82

LOD and LOQ: The LOQ's by this method were found to be as 7.42 and 0.026 ppm for DEC and LEVC respectively. Each value was verified by six individual injections of respective drug. The LOD's were found to be as 2.42 and 0.08 ppm for DEC and LEVC, respectively. These were confirmed by injecting respective standard drug solution at respective concentration for six times.

Ruggedness: 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 § Inertsil C18 (250×4.6 mm i.d., 5µ), Hypersil-BDS C18(250×4.6 mm i.d., 5µ) were used and the chromatograms were recorded.

TABLE 5: RUGGEDNESS RESULTS OF DEC AND LEVC

S. NO.	ANALYST-1				ANALYST-2			
Drug	DEC		LEVC		DEC		LEVC	
	Peak area	Cal. conc (ug/ml)	Peak area	Cal. Conc. (ug/ml)	Peak area	Cal. Conc. (ug/ml)	Peak area	Cal. Conc ug/ml
1.	2103.378	100.18	685.765	4.04	2107.750	100.39	683.679	4.03
2.	2087.765	99.76	689.125	4.06	2097.557	99.91	681.146	4.02
3.	2104.335	100.24	677.564	3.99	2089.672	99.88	688.256	4.05
4.	2099.528	100.01	680.298	4.01	2105.632	100.29	701.572	4.13
5.	2097.258	99.89	686.923	4.04	2096.598	99.83	678.832	3.99
Mean	2098.453	100.016	691.735	4.02	2099.442	100.06	691.897	4.04
SD	0.2		0.027		0.26		0.04	
%RSD	0.2		0.67		0.26		0.9	

Robustness: 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 (less than 2500 for both DEC&LEVC), resolution factor (15.873 for LEVC), Tailing factor (1.3 for DEC, 1.5 for LEVC)

TABLE 6: ROBUSTNESS FLOW RATE CHANGE RESULTS OF DEC AND LEVC

S.NO.	Peak area (at flow rate 0.9ml)				Peak area (at flow rate 1.1ml)			
	DEC	Conc.	LEVC	Conc	DEC	Conc.	LEVC	Conc
1.	2119.822	101.3	692.976	4.09	2100.057	100.06	712.826	4.2
2.	2118.667	101.2	694.481	4.10	2104.765	100.26	714.352	4.21
3.	2119.227	101.27	690.705	4.07	2103.776	100.20	709.695	4.18
4.	2117.767	101.2	693.962	4.09	2105.326	100.27	711.885	4.19
5.	2120.258	101.32	695.539	4.10	2102.943	100.16	713.256	4.2
Mean	2119.148	101.258	693.533	4.09	2103.373	100.19	712.403	4.196
SD	0.05		0.01		0.08		0.01	
%RSD	0.049		0.24		0.08		0.23	

TABLE 7: ROBUSTNESS WAVELENGTH CHANGE RESULTS OF DEC AND LEVC

S. NO.	Peak area (at wavelength 252nm)				Peak area (at wavelength 256nm)			
Drug	DEC	Conc.	LEVC	Conc.	DEC	Conc.	LEVC	Con
1.	2090.931	99.53	678.438	3.9	2178.927	104	551.144	3.25
2.	2091.767	99.53	673.894	3.97	2177.265	103.9	549.382	3.24
3.	2094.109	99.71	675.736	3.98	2177.176	103.9	554.476	3.27
4.	2092.842	99.59	677.649	3.99	2180.206	104	555.023	3.28
5.	2097.953	99.89	680.611	4.01	2179.953	104.1	558.194	3.3
Mean	2093.52	99.65	677.2656	3.97	2178.705	103.9	553.644	3.27
SD	0.15		0.04		0.08		0.02	
%RSD	0.15		1.0		0.07		0.6	

System Suitability: System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed. (Or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Resolution (R), Tailing factor (T) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of DEC and LEVC was validated or not. The results were shown

TABLE 8: SYSTEM SUITABILITY RESULTS OF DEC AND LEVC

Drug	Retention Time (min)	Peak area	USP Plate count	USP Tailing	Resolution
DIETHYLCARBAMAZINE CITRATE	2.060	2102.082	2727	1.3	-
CETRIZINE DIHYDROCHLORIDE	5.657	701.129	7879	1.5	15.873

Assay: Weigh not less than ten capsules. Accurately weigh and transfer powder equivalent to 58.9mg of sample into 50ml volumetric flask, to this 25ml of diluent was added, sonicated and made up to the volume and then filtered. About 10ml of the above solution was made up to 100 mL by using the same diluent.

Calculation:

$$\% \text{ Purity} = \frac{A}{A_S} \times \frac{Swt}{Sdwt} \times \frac{Avwt}{Lc} \times SdP$$

Assay of DEC:

TABLE 9: ASSAY RESULTS OF DEC

Sl. No.	Sample DEC	Peak Area	Cal. Amount (µg/ml)	Statistical Analysis	Standard	Peak Area	Cal. Amount (µg/ml)	Statistical Analysis
1.	Injection 1	2103.007	100.16	SD = 0.17 %RSD= 0.17	Injection 1	2102.082	100.12	SD= 0.15 %RSD= 0.15
2.	Injection 2	2099.756	99.92		Injection 2	2103.853	100.21	
	Average	2101.381	100.04		Injection 3	2098.958	99.91	
					Average	2101.631	100.08	

Assay of LEVC:

TABLE 10: ASSAY RESULTS OF LEVC

Sl. No	Sample: LEVC	Peak Area	Cal. Amount (µg/ml)	Statistical Analysis	Standard	Peak Area	Cal. Amount (µg/ml)	Statistical Analysis
1.	Injection 1	697.274	4.1	SD= 0.028 %RSD= 0.68	Injection 1	701.129	4.13	SD= 0.03 %RSD= 0.73
2.	Injection 2	702.614	4.14		Injection 2	696.579	4.1	
	Average	699.944	4.12		Injection 3	689.987	4.07	
					Average	696.232	4.08	

SUMMARY AND CONCLUSION: 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.

ACKNOWLEDGEMENTS: The authors thank the founder of JSS Mahavidyapeetha, Mysore and Dr. B. Suresh, Vice-chancellor, Mysore for providing the facilities to carry out this work.

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How to cite this article:
Movva KV, Jayalakshmi B, Amritharaj RV and Chilamkurthi S: A sensitive RP-HPLC method for simultaneous estimation of Diethylcarbamazine and Levo-Cetirizine in bulk and Pharmaceutical Dosage Form**.** *Int J Pharm Sci Res*, 2012; Vol. 3(9): 3347-3353.