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# A comparative study for estimation of Levocetirizine dihydrochloride in bulk drug and solid dosage formby reverse phase HPLC using C18 monolithic and C18 conventional column

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**Abstract:** Higher rate of sample analysis is one of the major demands in recent pharmaceutical analysis, mostly during product development of a new molecule as well as validation of industrial scale manufacturing process. The performance of monolithic HPLC column ChromolithTM (made by Merck, Germany) and conventional C18 particle packed column C18 Xterra column (made by Waters, USA) was tested and the amount of levocetirizine dihydrochloride in bulk drug and tablet dosage form was estimated. The analyte was eluted using simple mobile phase consisting acetonitrile and 0.05 M phosphate buffer (50:50 v/v) at pH 4.0 adjusted with orthophosphoric acid. The flow rate was set at 1.0 ml/ min and detection was carried out at 230 nm for both columns. The injection volume was set at 20 µL for conventional C18 column and 20 µL for monolithic column. The elution of analyte using monolithic column was found very rapid (Rt, 2.1 min) as compared with conventional C18 column (Rt = 5 min). The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, robustness and system suitability parameters in accordance with the ICH guideline Q2 (R1) [17]. This validated method showed that monolithic columns due to their high porosity nature can save analysis time with having great efficiency offered better results and was found to be suitable for high-throughput analysis as compared with conventional C18 column.
**Key words:** HPLC, Monolithic columns, Levocetirizine dihydrochloride, Validation.

## 1. Introduction

With recent advancements in the application areas of HPLC, the rate of analysis is becoming more and more important in fields like pharmaceutical analysis and toxicology laboratories, in order to achieve high throughput with reduced costs. HPLC, one of the most predominant techniques most widely used in pharmaceutical quality control, due to high sensitivity and selectivity of analytical method. However, the majority of HPLC based methods takes longer duration of time since several minutes (5-30 min) are required for a complete separation cycle. The primary reason behind this is that the conventional particulate based columns ( having particle size in the range of 3-5 µm) fail to operate at elevated flow rates ( > 2 mL/min) due to excessive back pressure. To overcome this, an interesting alternative method can be employed using monolithic columns. J.H. Knox and P.A. Bristow of Edinburgh University (Edinburgh, UK) recognized the potential advantages of monolithic columns more than 30 years ago [2]The recently invented monolithic columns offer new practical possibilities for decreasing retention times and/or increasing column efficiencies, while escaping the pressure constraint to a certain extent [3- 5]. Monolithic columns prepared from organic and silica

monomers offer very efficient separations at low back- pressure. Monolithic columns can potentially provide high performance close to that of UHPLC or CE using common HPLC instrumentation [6 - 9].

Levocetirizinedichydrochloride (LCZ), 2-[2-[4-[(R)-(4- chlorophenyl)-phenyl-methyl] piperazinyl-1-yl]ethoxy] acetic acid dihydrochloride, the R-enantiomer of racemic cetirizine, is is a third-generation nonsedative, potent, H1-antihistamine compound indicated for the treatment of allergic rhinitis and chronic idiopathic urticaria [10, 11].

Literature review reveals that some analytical methods have been reported for the determination of levocetirizine dihydrochloride in bulk drug and formulations by HPLC [12- 21]. There are some LC-MS/MS methods have been also reported for quantification of levocetirizine dihydrochloride in biological fluid [22 – 25].

The present work reports a comparative study for estimation of LCZ in bulk drug and solid dosage form tablets by RP-HPLC using C18 monolithic and conventional C18 column. The ability of the monolithic column to operate at high flow rates with low back pressure enabled the completion of the separation- detection cycle in 2 min.The assay method was validated as per ICH guidelines [26].

## 2. Experimental

### 2.1 Chemicals and Reagents

Working standard of Levocetirizine dihydrochloride (Purity 99.9 %) was procured from Hetero Labs Limited, Hyderabad, India. HPLC grade water, Acetonitrile, potassium dihydrogenphosphate, orthophosphoric acid used throughout this work and were provided by Merck-Millipore, India.

### 2.2 Instrumentation

A LC 2010 series (SHIMADZU) HPLC system equipped with a quaternary pump, column heater, auto-sampler, and UV detector was used. The data were collected and processed using Lab solution®software for all system suitability parameters.

### 2.3 Chromatographic conditions

Analysis was performed on a Xterra RP-18 column (5 $\mu$m particle size, 150 mm × 4.6mm) and on a Chromolith performance RP-18e columns (150× 4.6 mm, Merck). The mobile phase was degassed by sonication before use. The mobile phase consisting of a mixture of 0.5 M Phosphate buffer (adjusted pH 4.0 with *Orthophosphoric acid*) and Acetonitrile, 65:35 (v/v) and filtered through 0.45µm filter. The flow rate was 1 ml/min and columns temperature were maintained at 25ºC. The injection volume was 20 µl. The detection was carried out 230 nm with UV detection .Peak areas were used for signals evaluation, while each standard were injected five times and sample was in duplicate.The total run time was set at 10 min for conventional C18 column and 5 min for conventional column.

### 2.5 Preparation of Standard Stock Solution

Stock solution of LCZ was prepared by accurately weighted 50 mg of the drug and dissolved in the mobile phase to 100 mL volumetric flasks, working solutions (2-150 µg mL-1) were prepared by diluting appropriately the stock solutions with mobile phase.The stock solution and working solution were stored at 2–8 °C protected from light.

## 3. Validation of the method

Validation of optimized HPLC method parameters such as the specificity, linearity, precision, accuracy, and robustness were evaluated according to the ICH guidelines with respect to following parameters.

### 3.1 Linearity and range

The linearity was estimated by injecting six concentrations of the drug prepared in mobile phase and the final concentration range was 2 – 12 µg/mL ( n = 6 ). Six replicates of each concentration were injected to calculate

the linearity. The injection volume was 20 ul. The peak areas were plotted against the corresponding concentrations toobtain the calibration graphs.

### 3.2 Precision

Intermediate precision of the method was checked by repeating studies on three different days. Repeatability studies were performed by analysis of three different concentrations (2.5, 5, 10 µg/mL) of the drug in hexaplicate (n = 6) on the same day.

### 3.3 Limit of detection and limit of quantitation

LOD was considered as 3:1 ( signal: noise ) and LOQ as 10:1( signal: noise ). The LOD and LOQ were experimentally verified by diluting known concentrations of standard solution of LCZ until the average responses were approximately 3 or 10 times the standarddeviation of the responses for six replicate determinations.

### 3.4 Robustness of the method

To evaluate robustness of the HPLC method, few parameters were deliberately varied. Theparameters included variation of flow rate, percentage of methanol in the mobile phase, pH ofmobile phase. Robustness of the method was done at three different concentration levels 2.5, 7.5 and 10µg/mL for LCZ.

### 3.5 Specificity

The specificity of the method towards the drug was established through study of resolutionfactor of the drug peak from the nearest resolving peak in the presence of excipients.

### 3.6 Analysis of marketed formulation

To determine the content of LCZ in conventional tablets (Brand name: D-VENIZ,Sun Pharmaceuticals Industries Ltd., Batch No. SKK0179, label claim: 5 mg LCZ e pertablet, Expiry date: November 2012), the contents of twenty tablets were weighed, their meanweight determined and finely powdered. An equivalent weight of the powder/triturate wastransferred to a 50 mL volumetric flask containing 10 mL methanol, sonicated for 30 min anddiluted to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min.Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45µm filter (Millipore, Milford, MA). The above stock solution was further diluted to get samplesolution at three different concentrations of 2.5, 7.5 and 10µg/mL. A 20 µL volume of each samplesolution was injected into the LC, six times, under the conditions described above. The peak areaswere measured at 230 nm and concentrations in the samples were determined using multilevelcalibration developed on the same LC system under the same conditions using linear regressionequation.

### 3.6 Accuracy

Accuracy of the developed method was determined by applying the method to a drug sample(LCZ tablets) to which a known amount of LCZ standard powdercorresponding to 80, 100, and 120% of label claim were added (standard addition method). Thepercentage recoveries were calculated from the slope and Y-intercept of the calibration.

## 4. Results and Discussion

A variety of mobile phases were investigated in the development of a HPLC method for the analysis of LCZ in bulk drug and in tablet dosage form. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the assay. Theinitial method was developed with Xterra RP 18,150 x 4.6mm, 5 µm column using mobile phase as acetonitrile and potassium dihydrogen phosphate buffer in the ratio of 50:50adjusted pH 5.0 with triethylamine at a flow rate 1.0 ml per minute with isocratic elution. The injection volume used was 20µl and the runtime was 10 min for conventional column, and 5 min for chromolithic column [Fig. 1]. The method on the conventional column was found to be successfully transferable to the monolithic columns without modification [Fig. 2]. This showed that the selectivity of these two columns types is almostidentical.

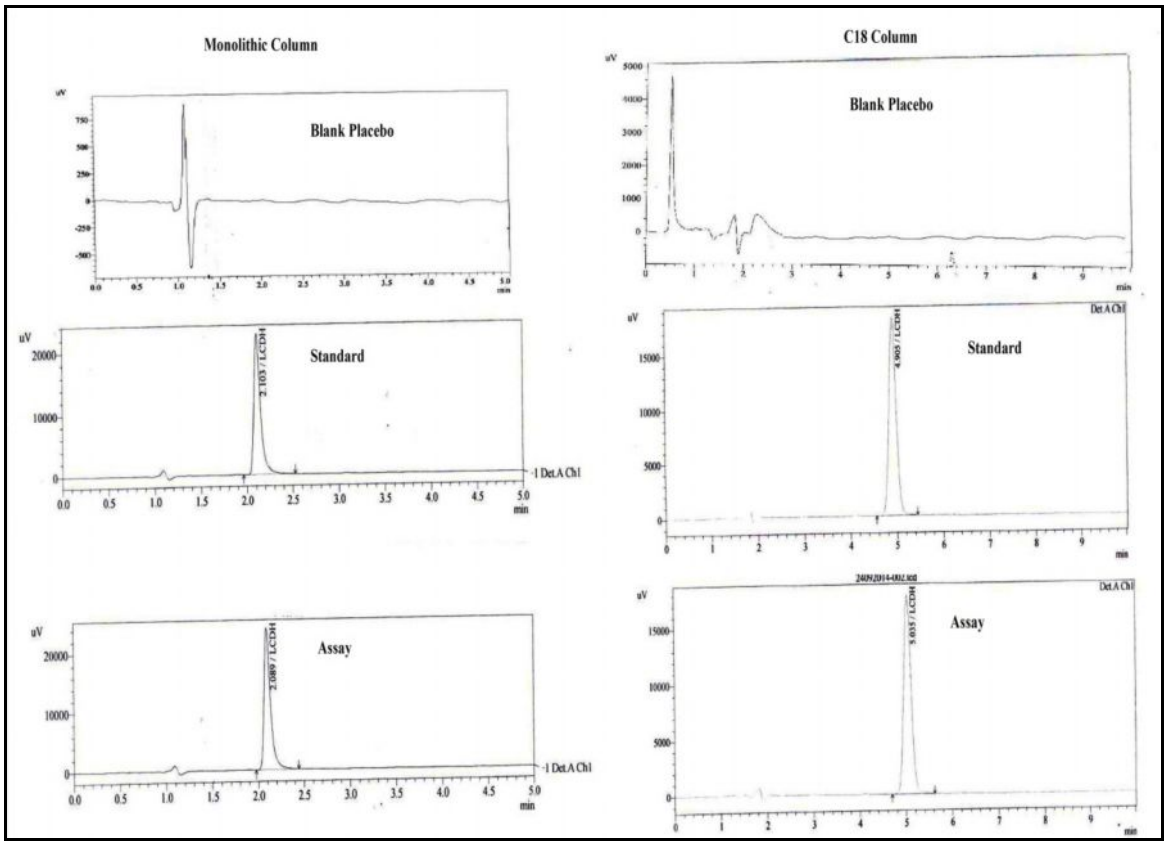


**Fig. 1 Comparative chromatogram of LCZ dihydrochlorideusing monolithic C18 and conventional C18 column**

These two methods have been validated as per ICH guidelines.To ensure assay precision, within day repeatability (n = 6) and between day repeatability (n = 6) were assessed for the conventional C18 column as well as for the monolithic column. For the precision studies, the within day RSD% equaled 0.67% for Conventional column and 0.66% for Monolithic column for peak area. The between days RSD % 0.48 % for Conventional column and 0.46% for Monolithic column for peak area. The linearity of calibration curves (peak area vs. concentration) for LCZ in the mobile phase were checked over the concentration range 50.02 – 150.60 µg/mL and correlation coefficients were about 0.99916and 0.99958 using conventional and monolithic columns, respectively. The accuracy of method was carried out from 50% to 150% in triplicate and the percent recovery of accuracy levels found to 99.1 % for conventional column and 100.2% for monolithic column. Robustness study, conducted by deliberate changes in pH of buffer, mobile phase composition and flow rate, revealed that there was no significant variation in system suitability parameters like retention time, theoretical plates and tailing factor. The comparative results of all validation parameters for conventional and monolithic are summarized in Table I.

**<Insert Table I>**

**Table I.**

| Components | System Suitability test % RSD | Precision | | | | Linearity |
|---|---|---|---|---|---|---|
| | | Repeatability (n= 6) | | Intermediate | | $R^2$ |
| | | Mean % assay | % RSD | Mean % assay | % RSD | |
| Conventional column | 0.67 | 99.4 | 0.66 | 99.99 | 0.49 | 0.99916 |
| Monolithic column | 0.66 | 99.8 | 0.55 | 100.23 | 0.44 | 0.99958 |

| Components | Recovery | | | | | |
|---|---|---|---|---|---|---|
| | At 50 % level (n = 3) | | At 100 % level (n = 3) | | At 150 % level (n = 3) | |
| | % recovery | % RSD | % recovery | % RSD | % recovery | % RSD |
| Conventional column | 99.85 | 0.08 | 99.02 | 1.49 | 98.41 | 0.05 |
| Monolithic column | 100.11 | 0.41 | 100.50 | 0.32 | 99.99 | 0.31 |

| Components | Robustness | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Temp. increased to 30°C | | Flow rate ± 0.1 mL/min | | Mobile phase variation ± 0.2 mL | | pH of Buffer ± 0.2 | |
| | $T_f$ | NTP | $T_f$ | NTP | $T_f$ | NTP | $T_f$ | NTP |
| Conventional column | 1.1 | 12541 | 1.01 | 11221 | 1.23 | 10891 | 1.29 | 10600 |
| Monolithic column | 1.09 | 9567 | 1.03 | 9125 | 1.12 | 8897 | 1.18 | 10120 |

| Component | System suitability Test | | | | | |
|---|---|---|---|---|---|---|
| | NTP | | Rt | | Tf | % RSD |
| Conventional column | 11547 | 5.02 | 1.05 | 0.04 | | |
| Monolithic column | 9872 | | | 2.10 | 1.10 | 0.34 |

## 4. Conclusion

Rapid high throughput dissolution and assay methods can is an growing industrial demand which can be achieved with the incorporation of a short monolithic column in an HPLC system for the dissolution study of Levocetrizine dihydrochloride containing formulations in place of conventional column. The monolithic column carried out the analysis less than 5 minutes with excellent analytical features ( viz linearity, precision, accuracy and selectivity). Higher assaying rate is extremely important advantage to work HPLC methods using particulate-based column, especially during new product development and validation of manufacturing process.

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