Estimation of simvastatin and cetirizine by RP-LC method: Application to freeze and thaw (FT) stability studies

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Abstract: Sensitive, simple, reliable and rapid HPLC technique for the estimation of simvastatin (SMV) and cetirizine has been designed in this study. The chromatographic conditions were set using Shimadzu LC-10 AT VP pump, with UV detector (SPD-10 AV-VP). System integration was performed with CBM-102 (Bus Module). Partitioning of components was attained with pre-packed C-18 column of Purospher Star (5 µm, 250 x 4.6 mm) at ambient conditions. Injected volume of sample was 10 µl. Mobile phase was composed of 50:50 v/v ratio of Acetonitrile/water (pH 3.0 adjusted with ortho-phosphoric acid) having 2 ml/minutes rate of flow. Compounds were detected in UV region at 225 nm. Percent Recovery of simvastatin was observed in the range of 98-102%. All results were found in acceptable range of specification. The projected method is consistent, specific, precise, and rapid, that can be employed to quantitate the SMV along with cetirizine HCl. It was estimated by 3 successive cycles of freeze and thaw stability. Results of FT samples were found within acceptable limits the method was developed and validated in raw materials, bulk formulations and final drug products.

Keywords: Simvastatin, Cetirizine, stability studies, HPLC determination.

INTRODUCTION

Cetirizine HCl (Fig 1) chemically defined as piperazine derivative 2-[2-,4-,4-chlorophenyl,phenylmethyl-piperazin-1-yl,ethoxy,acetic acid dihydro chloride), having molecular weight 461.8 (C₂₁H₂₇Cl₃N₂O₃), with is whitish or colorless compound having free solubility in aqueous medium, while completely insoluble in acetone and methylene chloride. It is reported to have antihistaminic activity as long acting compound and shown to produce mast-cell stabilization as well (Kuna et al., 2009). It is mostly prescribed in rhinitis and chronic urticaria for symptomatic relieving of hypersensitivity conditions (Ben-Chetrit et al., 2005). On the other hand, statins and other antihypertensive agents are successfully prescribed in treatment and prevention of cardiovascular abnormalities (Khalid et al., 2005). Numbers of methods are reported for in vitro estimation of simvastatins (Khalid et al., 2005; Ramakrishna et al., 2007 and Munir et al., 2014) and cetirizine (Paw et al., 2002; Jelińska et al., 2005 and Bajerski et al., 2005) in various samples of tablets, plasma/serum and raw material by chromatographic methods separately. Concurrent estimation of different compounds by HPLC method has obtained significant attention in past few years because of their significance in regular quality control timings.

Generally HPLC procedures require complicated and costly equipments, they need expensive solvents and also rigorous work is required for the preparation of samples. Simultaneous determination of a number of commonly co-administered drugs as diltiazem and statins (Sultana et al., 2010) lisinopril and statins (Sultana et al., 2011), prazosine and statins (Sultana et al., 2010), ceftriaxone sodium and statins (Sultana et al., 2010) and anti diabetic drugs with statins were also studied (Sultana et al., 2010), but not a single method for the instantaneous determination of cetirizine and simvastatin is available in literature. Hence, the objective of present study was to design a quick, precise, accurate, reliable, and economical HPLC method for the concurrent determination of simvastatin and cetirizine HCl, in raw materials, samples of bulk drug and final tablet, following ICH guidelines up to nano gram levels and thus it will be widely utilized in Pharmaceutical industries.

As current method also offers the advantages of low LOQ & LOD standards, it can be applied to biological samples estimation at nano scale limits as well (Sultana et al., 2010). Furthermore, this validated method can also apply to assess the probable in vitro interactions of cetirizine with simvastatin under simulating environments of human body using variable pH conditions. These methods could be applied for the quantitation of drugs as well as for clinical purposes.
MATERIALS AND METHODS

Instrumentation
Shimadzu HPLC composed of a ternary system of gradient (LC-10), pump (LC-10 AT), line degasser (DGU-14 AM), (Shimadzu Corporation Kyoto, Japan). C18, Purospher Star, column (stationary phase) was selected with general specification of 5 µm, 250 x 4.6 mm. Deionizer; Stedec (CSW-300)

Mobile phase and chromatographic parameters
Acetonitrile and water in a ratio of 50:50 v/v was used as mobile phase filtered (0.45µ) and degassed before experiment. Flow was adjusted with a rate of 2 ml min⁻¹ and detection wave length was 225 nm. Ambient temperature (25°C) conditions were maintained throughout the analyses. Sample injection volume was 20 µL. Recording and integration of chromatograms were performed on PC equipped with CLASS-GC (Version 5.03) software.

Chemicals and reagents
A reference standard of simvastatin was obtained from Geoofman Pharma (Pvt.) Ltd., and simvastatin tablets (Atcol 10mg) were procured. HPLC grade Acetonitrile and ortho-phosphoric acid (Merck, Germany).

Solution preparation Simvastatin standard stock solutions
Preparation of stock solution of standard simvastatin and cetirizine (100µgL⁻¹) were performed by dissolving 10 mg of each in 100mL flask, initial volume of diluent was 10mL followed by 30mL of mobile phase. Solubilization of compounds was facilitated by sonication, finally volume was made-up with mobile phase.

Working solution
Working solutions were prepared in the concentration range of 2.5-100 µg/mL⁻¹ for both (SMV and cetirizine) and stored at 20°C. These samples were also assessed for inter-day and inter-operator variability. A volume of 20 µL of each sample was injected and chromatogram was recorded.

Preparation of solution for tablet formulations
10 mg equivalent quantities of simvastatin and cetirizine were extracted from crushed sample (20 tablets) to determine the specificity and suitability of presented method. Samples were dissolved and diluted in 100mL flask with mobile phase separately. These solutions were filtered and further diluted to the required limit of concentrations to analyze the drug contents.

RESULTS

Chromatographic condition
Development of HPLC technique for the estimation of active compounds is considered to be very significant from past few years because of their efficient use in routine quality control assessment (Sultana et al., 2011; Sultana et al., 2011). In this investigation HPLC method development and optimization was performed using variable sets of parameters like composition of mobile phase, flow rate, and pH were varied to evaluate the optimized conditions of chromatographic operation. Primarily, ACN: H₂O in proportion of 50:50v/v was chosen as mobile phase, and run through Purospher Star, C18 (5µm, 250 x 4.6mm) column with a flow rate of 2 ml min⁻¹ using 225nm wavelength at UV range. Initially methanol: water as mobile phase was tested for determining the system suitability in different composition like 70:30, 80:20, 60:40 v/v, then acetonitrile and water having the above ratios was tried.

Fig.1: Structure of Simvastatin and Cetirizine.

Fig.2: Chromatogram of Cetirizine and Simvastatin in API.

Fig.3: Chromatogram of Cetirizine and Simvastatin in Formulation.
Mobile phase variations have resulted in substantial changes in the chromatographic conditions, which were observed through symmetry of peaks, retention time, and capacity factor. Optimum pH effect was found in relevance of peak sharpness and resolution at pH 3 for both constituents. Therefore, in the current study pH 3.0 was adjusted throughout and 225nm wavelength as isosbestic point was used. During the optimization phase, peaks were also resolved sharply when the same mobile phase was adjusted to pH 2.8 at a flow rate of 2 mL/min^-1. For simultaneous estimation of simvastatin, and cetirizine, individual samples of drugs were introduced into the injector at the concentration of 100µg/mL. Elution and resolution pattern were premeditated for both moieties. Data of system suitability is presented in table 1. Cetirizine and SMV time of retention was found to be 3 and 6 minutes respectively, which was in accordance of resolution criteria given in USP 2008. Test solution chromatogram is presented in figs. 2-3.

**DISCUSSION**

**Method validation parameters**

Validation of given method was performed in raw materials, drug products and in serum. For this purpose, ICH (International Conference on the Harmonization) guidelines recommended for technological requirements for pharmaceutical products registration devised for human Use. In this connection, system suitability, selectivity, sensitivity, range/linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) (Lister, 2005) were measured.
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Selectivity and specificity
Peak resolution factor of SMV and cetirizine (IS) was established to study the selectivity (specificity) of the proposed method (table 1). No interference of excipients was found and method has shown good resolutions (fig. 2).

System suitability
All parameters of system suitability were found within accept table limits (ICH, 2003) and summarized in table 1.

Linearity
Calibration curves were constructed by plotting several injected concentrations against the peak area (Sultana et al., 2011), the calibration curves were found to be in the range of 2.5, 5, 10, 25, 50 and 100µg/ml for simvastatin and shown linear pattern ($r^2=0.999$). Table 2 shows the regression statistics of cetirizine and simvastatin.

Accuracy and precision
The recovery data of spiked placebo samples was used to determine the method accuracy. Sui table fractions of stock samples of SMV were spiked with placebo matrix (blank) to fabricate 80, 100 and 120% of the hypothetical concentration. Sample (SMV) mean recovery was found to be 100% (table 3). Six replicates of standard solutions were used for precision determinations and values of relative standard deviations (RSD) for SMV were observed <2%. Individual sample triplicates were injected and mean responses (peak area) were estimated. Inter-day and inter-instruments results were analyzed to assess the intermediate precision (table 4).

Freeze and thaw (FT) stability
It was estimated by 3 successive cycles of freeze and thaw. Each cycle comprises of 5 samples of replicated concentration. Samples were stored for the period of 24 hours (-20°C). Thawing of samples (n=5) was performed while remaining was refrozen for 24hrs. Same protocol was repeated for rest of the cycles (FT-2 & FT-3). Results of FT samples were compared with Fresh samples of similar concentrations with respect to mean, SD and % CV and recovery. Results of FT samples were found within accept table limits (table 5). Kozikowski et al. determined the sample stability in DMSO using HPLC method to characterize the influence of freeze/thaw on compound veracity.

Robustness and ruggedness
In this study method reproducibility was calculated by applying it to varied instrumental settings Sultana et al., 2011; Arayne et al., 2012). Method robustness was evaluated by altering the mobile phase composition ±5 v/v, varying the pH ±2 and by adjusting the flow rate ±0.2 ml min$^{-1}$. Results were found to be within adequate limits.

CONCLUSION
This projected RP-HPLC technique was found suitable for the estimation of cetirizine and simvastatin in raw materials and formulations. Biological sample applications can further assessed with this method at sensitive scale level. This method was simple, precise, economical and robust.

REFERENCES


Table 5: Freeze and thaw stability of simvastatin and cetirizine

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<tr>
<th>PARAMETERS</th>
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<td><strong>Cetirizine</strong></td>
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Note: FT Cycle = freeze and thaw cycle

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ICH guideline Q2B; Validation of Analytical Procedures; Methodology (2003).


