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

Safila Naveed[1], Khan Usmanghani[1,4], Aisha Sana[1,5], Huma Ali[2], Farya Zafar[3], Fatima Qamar[1], Ghulam Sarwer[1], Sarah Abbas[1], M Tanweer Alam[1] and Muhammad Ibrar Shinwari[6]

[1]Jinnah University for Women, Karachi, Pakistan
[2]Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan
[3]Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[4]Herbion Pakistan (Pvt.) Ltd., Karachi, Pakistan
[5]Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
[6]Department of Environmental Sciences, Faculty of Basic & Applied Sciences, International Islamic University, H-10, Islamabad, Pakistan

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 accept table 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 accept table 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 ($C_{21}H_{27}Cl_3N_2O_3$), 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 tings. 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), ceftriaoxone 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.

**Corresponding author:* e-mail: safila117@yahoo.com

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 iltered (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 Geofman 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 (100µgmL^{-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 µgmL^{-1} for both (SMV and cetirizine) and stored at 20ºC. 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: H_2O 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.

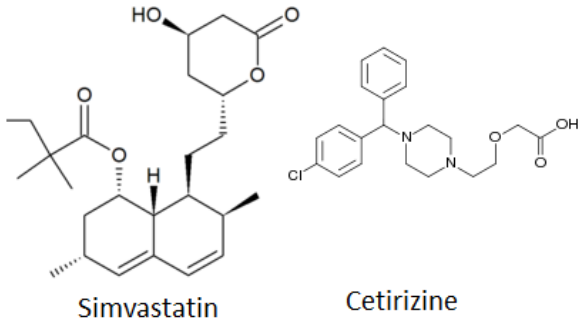

Fig. 1: Structure of Simvastatin and Cetirizine.

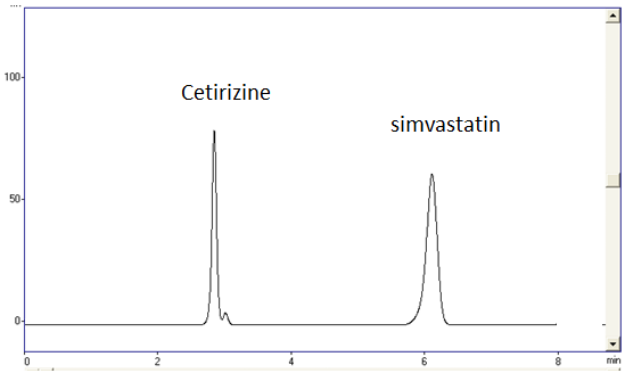

Fig. 2: Chromatogram of Cetirizine and Simvastatin in API.

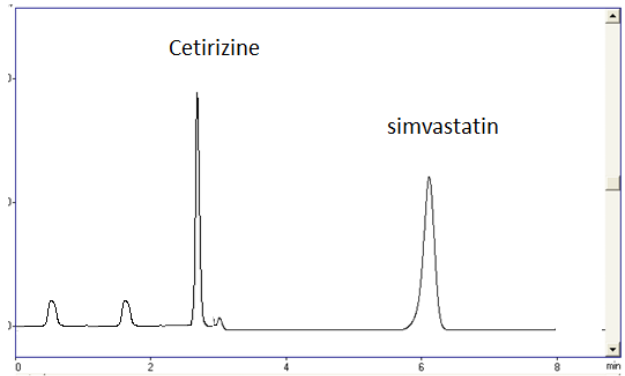

Fig. 3: Chromatogram of Cetirizine and Simvastatin in Formulation.

Table 1: System suitability parameters

Parameters	Retention time (Rt)	Capacity factors (K')	Theoretical plates (N)	Tailing factor (T)	Resolution (R)	Separation factor
Simvastatin	0.510	0.12	7200	0.87	0.45	0.55
Cetirizine	0.550	0.13	7250	0.78	0.47	0.35

Table 2: Regression equations with LOD, LOQ

Drugs	Regression equations	LOD ng/mL	LOQ ng/mL	r^2
Simvastatin	y = 17339x - 1480.9	0.2	0.9	0.9995
Cetirizine	y = 15930x - 70192	0.8	2.5	0.9954

LOD=(Limit of detection), LOQ=(limit of quantification) Correlation coefficient (r^2)

Table 3: Accuracy and % Recovery studies of Simvastatin and Cetirizine

Drugs	Conc%		API		Formulations
		%RSD	% Recovery	%RSD	% Recovery
Simvastatin	80%	0.44	100.61	0.19	100.98
	100%	0.32	100.59	0.24	100.01
	120%	0.11	100.20	0.38	100.15
cetirizine	80%	0.16	100.98	0.43	99.95
	100%	0.25	100.70	0.14	100.24
	120%	0.46	100.55	0.06	100.51

Table 4: Inter day and intraday precision of Simvastatin and Ceterizine

Drugs	Conc. Injected μg mL^{-1}	Inter-day		Intra-day	
		%RSD	%Recovery	%RSD	%Recovery
Simvastatin	0.625	0.73	100.23	0.695	99.98
	1.25	0.34	100.62	0.523	98.53
	2.5	0.37	101.55	0.416	100.91
	6.25	0.25	102.31	0.522	100.14
	12.5	0.52	100.26	0.148	99.41
	25	0.22	101.63	0.742	99. 82
Cetirizine	0.625	0.571	99.89	0.683	100.04
	1.25	0.453	100.43	0.496	101.76
	2.5	0.427	100.45	0.426	100.25
	6.25	0.292	101.67	0.364	100.37
	12.5	0.482	101.25	0.249	99.99
	25	0.227	101.62	0.536	100.35

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 $mLmin^{-1}$. For simultaneous estimation of simvastatin, and cetirizine, individual samples of drugs were introduced into the injector at the concentration of 100μgmL^{-1}. Elution and resolution pattern were premeditated for both moieties. Data of system suitability is presented in table 1. Cetrizine 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.

Table 5: Freeze and thaw stability of simvastatin and cetirizine

FREEZ AND THAW STABILITY				
Simvastatin			1.25 (µg/ml)	
PARAMETERS	FRESH SAMPLE	FT CYCLE 1	FT CYCLE 2	FT CYCLE 3
MEAN(n=5)	1.25	1.248	1.247	1.2462
SD	0.00122	0.00122	0.00141	0.00130
CV%	0.0980	0.0981	0.1134	0.1046
% RECOVERY	100.000	99.840	99.760	99.696
Cetirizine			3 (µg/ml)	
CODE	FRESH SAMPLE	FT CYCLE 1	FT CYCLE 2	FT CYCLE 3
MEAN(n=5)	3.01744	2.9932	2.987	2.9832
SD	0.04783	0.00976	0.01198	0.01256
CV%	1.5852	0.3260	0.4010	0.4210
% RECOVERY	100.5813	99.77333	99.56667	99.44

Note: FT Cycle = freeze and thaw cycle

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 and100µgmL^{-1} 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

Arayne MS, Sultana N, Tabassum A, Ali SN and Naveed S (2012). Simultaneous LC determination of rosuvastatin, lisinopril, captopril, and enalapril in API, pharmaceutical dosage formulations and human serum. *Med. Chem. Res.,* **21**(12): 4542-4548.

Bajerski L, Cardoso SG, Diefenbach IF, Malesuik MD, Sílvia H and Borgmann M (2005). Liquid chromatographic determination of cetirizine in oral formulations. *J. AOAC Int.,* **88**(2): 424-427.

Ben-Chetrit E, Amir G and Shalit M (2005). Cetirizine: An effective agent in Kimura's disease. *Arthritis Rheum.,* **53**(1): 117-118.

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

Jelińska A, Stanisz B, Zajac M, Musiał W, Ostrowicz A (2005). Determination of cetirizine dichloride in tablets by HPLC method. *Acta. Pol. Pharm.,* **57**(3): 171-173.

Khalid MP, Muzeeb M, Jafar SS, Basha SD, Ramesh M and Srinivas NR (2005). Analysis of five HMG-CoA reductase inhibitors atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin: Pharmacological, pharmacokinetic and analytical overview and development of a new method for use in pharmaceutical formulations analysis and *in vitro* metabolism studies. *Biomed. Chromatogr.,* **20**(3): 282-293.

Kozikowski BA, Burt, Tirey DA, Williams LE, Kuzmak BR, Stanton DT, Morand KL and Nelson SL (2003). The effect of freeze/thaw cycles on the stability of compounds in DMSO. *J. Biomol. Screen.,* **8**: 210-215.

Kuna P, Bachert C and Nowacki Z *et al* (2009). Bilastine International Working Group. Efficacy and safety of bilastine 20 mg compared with cetirizine 10 mg and placebo for the symptomatic treatment of seasonal allergic rhinitis: A randomized, double-blind, parallel-group study. *Clin. Exp. Allergy,* **39**(9): 1338-1347.

Lister AS (2005). Validation of HPLC Methods in Pharmaceutical Analysis. Elsevier Inc., UK, pp.197-211.

Munir A, Ahmad M, Malik MZ and Minhas MU (2014). Analysis of Simvastatin using a Simple and Fast High Performance Liquid Chromatography-Ultra Violet Method: Development, Validation and Application in Solubility Studies. *TJPR.,* **13**(1): 135-139.

Paw B, Misztal G, Hopkała H and Drozd J (2002). Development and validation of a HPLC method for the determination of cetirizine in pharmaceutical dosage forms. *Pharmazie*., **57**(5): 313-315.

Ramakrishna N, Koteshwara M and Vishwottam K (2007). Chromatography-mass spectrometry methods for the quantitation of statin in biological samples. *J. Pharm. Biomed. Anal.,* **44**: 379-387.

Sultana N, Arayne MS and Naveed S (2010). Simultaneous Determination of Captopril and Statins in API, Pharmaceutical Formulations and in Human Serum by RP-HPLC. *J. Chin. Chem. Soc.,* **57**: 378-383.

Sultana N, Arayne MS and Naveed S (2011). Simultaneous determination of Enalapril and Statin's in Pharmaceutical formulations by RP-HPLC. *J. Chil. Chem. Soc.,* **56**: Nº 2.

Sultana N, Arayne MS and Naveed S (2011). Validated method for the simultaneous determination of lisinopril, pravastatin, atorvastatin and rosuvastatin in API, formulations and human serum by RP-HPLC. *Chinese J. Chem.,* **29**: 2421-2427.

Sultana N, Arayne MS and Naveed S (2011). Validated Method for the Simultaneous Determination of Lisinopril, Pravastatin, Atorvastatin and Rosuvastatin in API, Formulations and Human Serum by RP-HPLC. *Chin. J. Chem.,* **29**: 1216-1220.

Sultana N, Arayne MS and Naveed S (2011). Validated Method for the Simultaneous Determination of Lisinopril, Pravastatin, Atorvastatin and Rosuvastatin in API, Formulations and Human Serum by RP-HPLC. *Chin. J. Chem.,* **29**(6): 1216-1220.

Sultana N, Arayne MS and Shehzad W (2010). Simultaneous Determination of Ceftrioxazone sodium and Statin Drugs in Pharmaceutical Forms and Human Serum by RP-HPLC. *J. Chil. Chem. Soc.,* **55**(2): 193-198.

Sultana N, Arayne MS, Merza AZ and Shamshad H (2010). High-performance liquid chromatographic analysis of pioglitazone, gliquidone, rosuvastatin and simvastatin in formulations and human serum *Chin. J. Chem*., **28**(10): 1998-2002.

Sultana N, Arayne MS, Shafi N, Siddiqui FA and Hussain A (2010). Development of a RPHPLC method for the simultaneous analysis of diltiazem and statin: Application in pharmaceuticals and human serum. *Anal. Methods,* **2**: 1571-1576.

Sultana N, Arayne MS, Shah SN, Shafi N and Naveed S (2010). Simultaneous determination of prazosine, atorvastatin, rosuvastatin and simvastatin in API dosage formulations and human serum by RP-HPLC. *J. Chin. Chem. Soc.,* **57**(6): 1286-1292.

Thammera RK, Nikhil RS, Pasikanti KK, Menon CA, Venkata PK, Ramesh M and Srinivas NR (2006). Determination of rosuvastatin in rat plasma by HPLC: validation and its application to pharmacokinetic studies. *Biomed. Chromatogr.,* **20**(9): 881-887.