International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF OLMESARTAN IN BULK DRUG BY REVERSE PHASE LIQUID CHROMATOGRAPHY METHOD

Amit Asati[1]*,
Anita Shinde[2], Suman Malik[1], K.C. Asati[1]

[1]Department of chemistry sadhu vaswani college bairagarh, Bhopal, India
[2]Department of chemistry i. E. H. E, Bhopal, India

ABSTRACT
A simple, precise and reversed phase liquid chromatographic method was developed and validated for estimation of olmesartan in bulk drug. It is an angiotensin II receptor antagonist, Angiotensin-II is a substance produced in the body which causes blood vessels to tighten. It blocks the action of angiotensin-II and therefore relaxes your blood vessels. This helps lower your blood pressure. The separation of olmesartan with its known impurities was achieved on C-18 1.7µ, (2.1 X 100) mm analytical column with mobile phase consisted of buffer (0.05% TFA in water, adjust pH 6.2 with dilute ammonia) : acetonitrile (20:80v/v) at isocratic flow of 0.30ml/min with UV detector 210 nm wavelength and 3µl of sample volume was injected. The method was successfully validated in accordance to ICH guidelines and usp pharmacopeia for accuracy, precision, specificity, linearity. The linear regression analysis data for calibration plots showed good linear relationship correlation factor is more than 0.999 in the concentration range 50-150µg/mL for olmesartan. The % accuracy was within the range between 98% and 102%. The percentage RSD for precision method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of olmesartan in bulk samples.

Key words: *Accuracy, ICH, Isocratic, linearity, olmesartan, Precision, RP-LC, validation, USP pharmacopeia*

Correspondence to Author

Amit Asati

Department of chemistry sadhu vaswani college bairagarh, Bhopal, India

Email: amit.asati29@gmail.com

INTRODUCTION

Olmesartan is an angiotensin II receptor antagonist (more commonly called an "ARB", or angiotensin receptor blocker), Angiotensin-II is a substance produced in the body which causes blood vessels to tighten. It blocks the action of

angiotensin-II and therefore relaxes your blood vessels. This helps lower your blood pressure. It is used alone or in combination with other antihypertensive agents. Olmesartan is chemically described as a 4-(1-Hydroxy-1-methylethyl)-2-propyl-1-[[2'-[1-(triphenylmethyl)-1H-tetrazol-5-yl][1,1'-biphenyl]-4-yl]methyl]-1H-imidazole-5 carboxylic acid (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester. Its empirical formula is C28H44N6O6, and the structural formula is shown in **figure-I**. Olmesartan is a white to off-white crystalline powder with a molecular weight of 800.91g/mol. Olmesartan is slightly soluble in alcohol and methylene chloride and practically insoluble in water.[1-4]

OBJECTIVE

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Validation is the process of generating experimental data that provides evidence that the performance of an analytical method is adequate for reliably assessing product quality.[5-8] The validation procedure has been performed by using ultra performance liquid chromatography. The method has been validated for linearity, precision (system repeatability, method repeatability, and method reproducibility), accuracy, range, specificity, and solution stability.[9-11] Literature survey indicates that there is no short run time LC method available for assay determination of olmesartan [12-15] thus we aimed to develop it. Liquid chromatography is a new technique used in analytical chemistry for separating and analyzing substances. Chromatography depends on the distribution of the mixture between two phases, one of them is fixed and is called Stationary phase while the other is not fixed and is called the Mobile phase. The mixture is dissolved in the moving phase and passed over a stationary phase. When a mixture of components is introduced in to a LC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated [15-21].

Medical options, several classes of drugs are used for treatment of Hypertension. Actually now days cost of medicines are too much high. There are two major ways to reduce the cost of medicine.

1) By route of synthesis 2) By analysis cost

Here an attempt has been made to reduce the cost of medicine trityl candesartan (anti-hypertensive drug) by reducing the analysis cost and develop such type of analytical method in which

1) There is Minimum solvent consumption,
2) Reduced analysis time
3) Chemicals and reagents which are used in the method are cheap and easily available.

Thus purpose of my research work to develop the analytical method for olmesartan anti-hypertensive pharmaceutical drugs by liquid chromatography and validate these methods with the guidance of United state Pharmacopeia (USP) and International Conference on Harmonization (ICH).

EXPERIMENTAL

Chemical and reagents:- Pure samples of olmesartan were obtained as gift. LC grade Acetonitrile, Trifluoroacetic acid and ammonia were purchased from Merck Company Mumbai. High purity deionised water was obtained from [Millipore, Milli-Q] purification system.

Instrumentation and chromatographic conditions:- The analysis of the drug was carried out on a waters acquity UPLC (Ultra performance liquid chromatography) binary gradient system. Acquity BEH C-18 1.7µ, (2.1 X 100) mm, Make: Waters, analytical column was used for separation. Mobile phase consisted of buffer (0.05% TFA in water adjust pH 6.2 with dilute ammonia) : Acetonitrile (20:80 v/v). Mix well and filter through 0.22µm filter. The mobile phase was prepared freshly and degassed by sonicating for 5min before use. Water : Acetonitrile (50:50 v/v) was used as diluents. The analysis was done on isocratic flow of 0.30ml/min with UV detection wavelength was performed at 210 nm at 30°C temperature using 3.0 µL injection volumes with auto injector.

Standard solution and sample solution preparation:- Accurately weigh and transfer 20mg of Olmesartan working standard and sample into separate 20mL volumetric flask, add about 15mL of diluent in each volumetric flask and sonicate to dissolve it completely and make volume up to the mark with the same. Further pipette out 5mL of the above stock solution into a 50mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.22µm filter. Obtain both solutions concentration were 100µg/ml. These solutions were injected into LC system. To determine, peak area of olmesartan was measured. Calculate % olmesartan by following formulae.

Calculation

$$(\%)\ \text{olmesartan} = \frac{A_1 \times C_2}{A_2 \times C_1} \times P$$

Where,

A_1 = Area of Olmesartan in sample

A_2 = Area of Olmesartan in standard

C_1 = Concentration of Olmesartan in sample (mg/ml)

C_2 = Concentration of Olmesartan in Standard (mg/ml)

P = Potency of Standard

RESULT

Method validation:- The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability.

Linearity:- The linearity of an analytical procedure is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. It should be established initially by visual examination of a plot of signals as a function of analyte concentration of content. If there appears to be a linear relationship, test results should be established by appropriate statistical methods (e.g., by calculation of a regression line by the method of least squares).

Five standard solutions of Olmesartan were prepared from three stocks in the range of 50% to 150 % of the nominal concentration and injected once. Linearity regression analysis demonstrated the acceptability of the method for quantitative determination of Olmesartan over the concentration range of about 50ppm to 150ppm of the nominal concentration. Linearity graph was shown in **figure-2** and slope, intercept correlation factor and Regression equation were shown in **table-I**

Precision:-The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations and three replicates of each concentration, or a minimum of six determinations at 100% of the test concentration).

System Repeatability:- Standard solution is prepared 100ppm were injected in six times and RSD of areas and retention times were calculated. The percentage RSD of areas was less than 2.0% and the % RSD of retention times was less than 5.0 %. Result are presented in **table-2**

Method repeatability:- Six preparation of olmesartan sample was analyzed from sample preparation to final results by the same analyst and the percentage RSD of obtained results was less than 2% and obtained results were within given range 100 ± 2. Result are presented in **table-3**

System Reproducibility:- Three olmesartan sample are analysed by this method in duplicate preparation and obtain results are in **table-4**

Accuracy:- It is defined as the closeness of test results obtained by the method to the true value. It may often be expressed as percent recovery by the assay of known, added amounts of analyte. Accuracy is a measure of the exactness of the analytical method. The ICH documents recommend

that accuracy be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration). The three different concentrations of olmesartan standard solutions were determined from three replicate injections, using the linear regression lines (linearity section). The deviations of the obtained results (expressed as percentage accuracy) were calculated from the true values were presented in **table-5.** The average deviations from true value are less than 2.0 %.

Specificity:- The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients. The specificity of the method was verified by testing the blank, standard and sample (un-spiked and spiked), determined the resolution factors between analyte peak (Olmesartan) and the nearest peak.

No significant interfering peak appeared in the blank, System suitability and standard chromatogram at the retention times of the analyte peaks.

Range:- The range obtained from Linearity, Precision and Accuracy is summarized ibesartan-50ppm to 150ppm (50% to 150% of nominal sample concentration)

Olmesartan Concentration(ppm)	Olmesartan Area
47.13ppm	2305711
75.42ppm	3549462
94.73ppm	4417765
105.19ppm	5024461
121.88ppm	5815602
Slope	**47044117**
Intercept	**41707**
Correlation factor	**0.998**

Table-1 Linearity Data

System Repeatability		
Concentration (ppm)	**Retention time (min)**	**Area**
94.74	3.084	4490020
94.74	3.088	4441735
94.74	3.092	4413386
94.74	3.096	4419402
94.74	3.099	4419413
94.74	3.101	4429113
Average	**98.3**	**4435511.5**
STDEV	**0.01**	**28492.3**
%RSD	**<0.1**	**0.6**

Table-2 System Repeatability data

Method Repeatability			
Concentration (ppm)	**Retention time (min)**	**Area**	**% Olmesartan**
94.50	3.103	4417765	98.4
94.69	3.105	4406626	98.0
93.99	3.108	4414731	98.9
94.93	3.108	4413246	97.9
93.85	3.111	4404541	98.8
94.46	3.108	4397132	98.0
Average	**3.107**		**98.3**
STDEV	**0.003**		**0.437**
%RSD	**<0.1**		**0.4**

Table-3 Method Repeatability data

Method Reproducibility				
S No.	**Concentration (ppm)**	**Area**	**% Olmesartan**	**% Olmesartan Average**
Sample-I Pre-I	94.93	4334348	96.1	95.8
Sample-I Pre-II	95.25	4318207	95.4	
Sample-II Pre-I	93.89	4310631	96.6	96.2
Sample-II Pre-II	94.69	4309695	95.8	
Sample-III Pre-I	94.13	4292437	96.0	95.8
Sample-III Pre-II	94.78	4301411	95.5	

Table-4 Method Reproducibility data

Injection No	**Level**	**Concentration(ppm)**	**Area**	**Calculated concentration (ppm)**	**Accuracy (%)**
1	80 %	75.42	3549462	74.563	98.87
2			3566120	74.917	99.34
3			3549222	74.558	98.86
Average			**3554935**		**99.0**
1	100 %	94.74	4490020	94.556	99.81
2			4441735	93.530	98.72
3			4413386	92.927	98.09
Average			**4448380**		**98.9**
1	120 %	105.19	5024461	105.92	100.69
2			5016960	105.76	100.54
3			5017345	105.77	100.55
Average			**5019589**		**100.6**

Table-5 Accuracy test data of Olmesartan

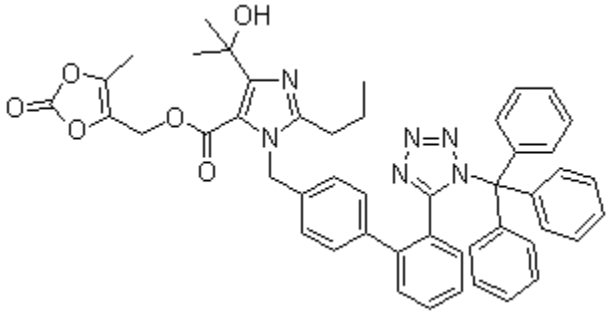


Figure-1 Olmesartan Structute

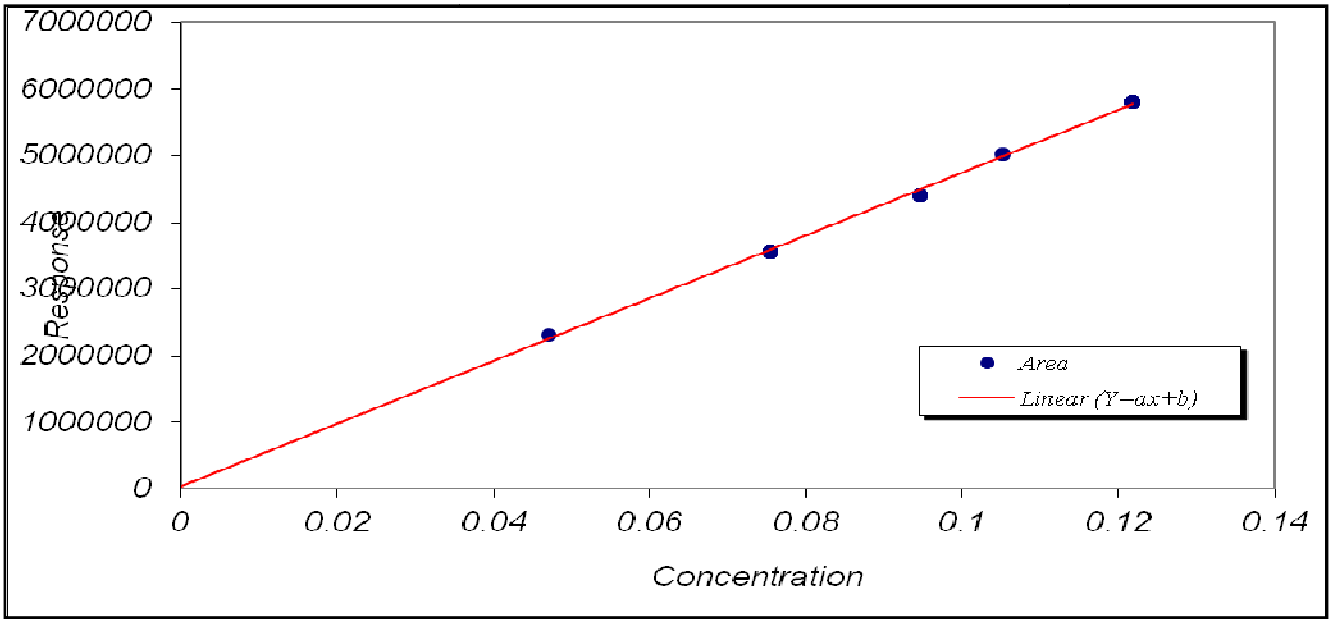


Figure-2 Linearity graph

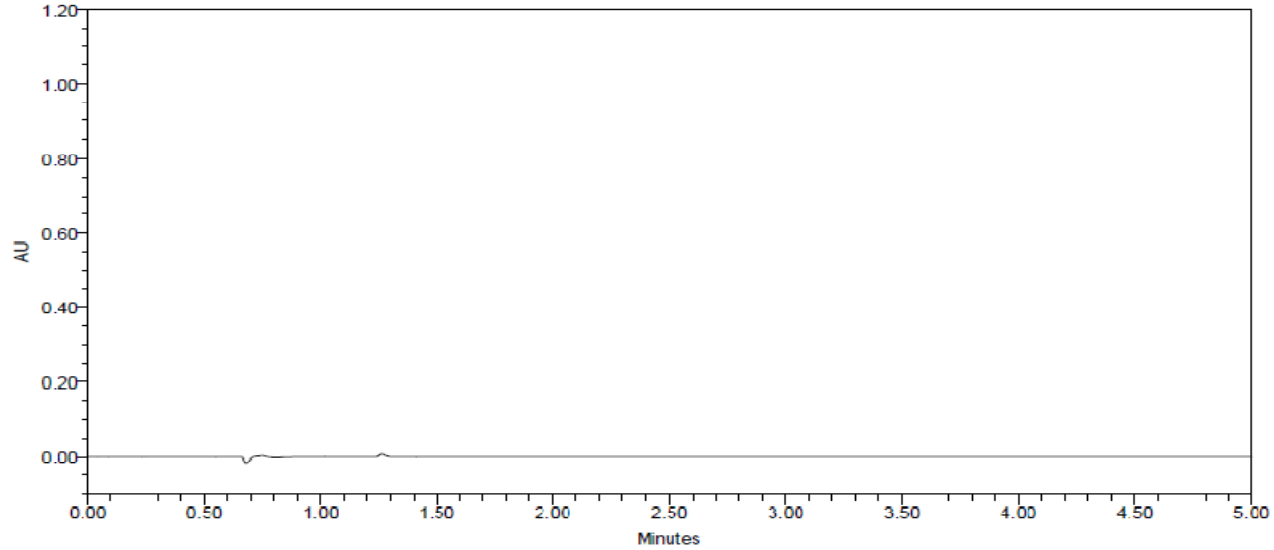
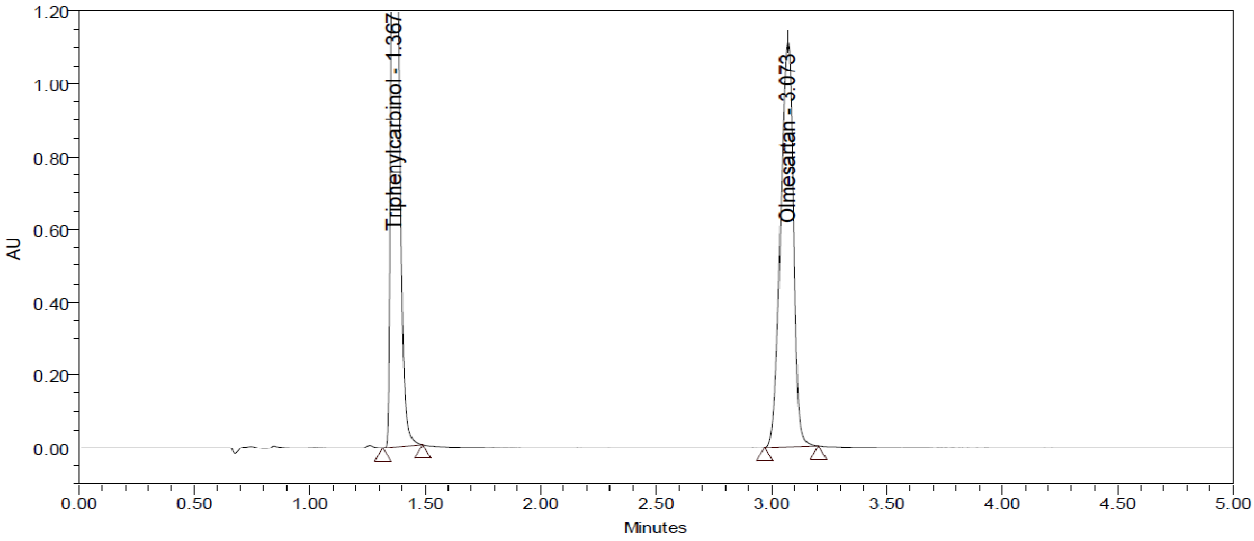


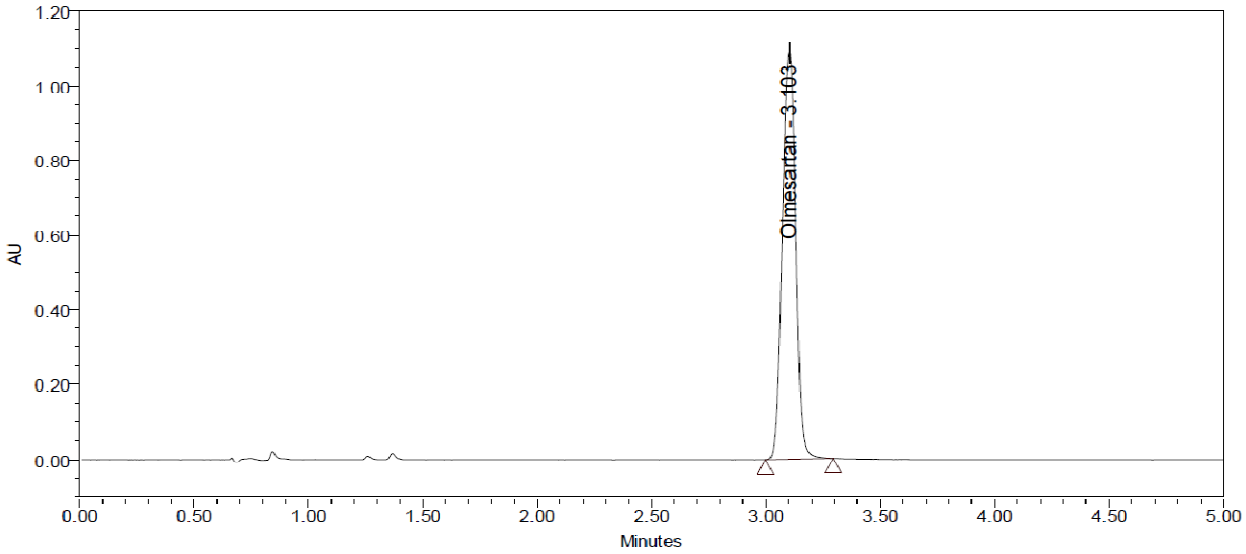
Figure-3 Typical Blank Chromatogram



Peak Results

	Name	RT	Area	% Area	RT Ratio	Resolution	USP Tailing
1	Inphenylcarbinol	1.367	4150482	48.88	0.44		1.47
2	Olmesartan	3.073	4340253	51.12	1.00	19.1	0.88
Sum			8490735.2				

Figure-4 Typical System Suitability Chromatogram



Peak Results

	Name	RT	Area	% Area
1	Olmesartan	3.103	4417765	100.00
Sum			4417765.5	

Figure-5

Typical Standard Chromatogram (100ppm)

DISCUSSION

The method validation demonstrated that The Method "Determination of Olmesartan Assay content by liquid Chromatography is selective, precise, linear, and accurate for performing the determination over the required concentration ranges of 50 to150 % of olmesartan nominal sample concentration so it should be applied routine analysis of determination of candesartan in bulk drug.

ACKNOWLEDGEMENT

All authors are grateful for principal of sadhu vaswani college, bairagarh Bhopal for providing research centre where research work was carried out. Thanks to all faculty of this institute for giving all possible facilitates encouragement. Finally thanks to our family for giving moral support and confident.

REFERENCES

1. The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals, Thirteenth Edition, Merck & Co. Inc., 2001, 13:3453.
2. Wu L, Zhang Z, Tian Y, Li W, Xu F, Chen Y, Wei H (2005) Determination of lafutidine in human plasma by high-performance liquid chromatography-electrospray ionization mass spectrometry: application to a bioequivalence study. J Mass Spectrom. 40(12):1637–1643
3. L Gonzalez; RM Alonso; RM Jimenez. Chromatographia., 2000, 52(11-12), 735-740.
4. E Caudron; S Laurent; EM Billaud; P Prognon. J-Chromatogr,-B:-Anal-Technol-Biomed-Life-Sci., 2004, 801(2), 339-345.
5. USP 32 <1225>: "Validation of Compendial Methods".
6. Guidance for Industry – Analytical Procedures and Method Validation (August-2000).
7. W.D. Snyder, L. Blumberg, in: P. Sandra, M.L. Lee (Eds.), Proceedings of the 14th International Symposium on Capillary Chromatography, Baltimore, MD, May 1991, p. 28
8. WHO, Guidelines for Stability Testing of Pharmaceutical Products Containing Well Established Drug Substances in Conventional Dosage Forms, in WHO Expert Committee
9. Willard Hobart. H., Merritt L.L., Dean John.A., Instrumental Methods of Analysis, 7th edition, CBS Publishers, 580-610.
10. International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human use, ICH harmonized tripartite Guideline, Validation of Analytical procedures Text and methodology Q2 (R1), 2005.
11. R. Jenke, “Chromatographic Method Validation: A review of Current Practices and Procedures. I. General Concepts and Guidelines”, J. Liq. Chrom. and Rel. Technol., vol. 19 (1996), pp. 719-736.
12. David M. Bliesner, Validating Chromatographic Methods, (John Wiley and Sons,2006, p. 72).
13. J Nie; M Zhang; Y Fan; Y Wen; BG Xiang; YQ Feng. J-Chromatogr,-B:-Anal-Technol-Biomed-Life-Sci., 2005, 828(1-2), 62-69.
14. AK Shakya; YM Al-Hiari; OM Alhamami. J-Chromatogr,-B:-Anal-Technol-Biomed-Life-Sci. 2007, 848(2), 245-250.
15. RT Sane; M Francis; S Pawar. Indian-Drugs., 2002, 39(1), 32-35.
16. Ramzia I. El-Bagary, Hanaa M. Hashem, Waleed A. Ebeid J. Chem. Pharm. Res., 2011, 3(4): 722-733
17. K. Balamuralikrishna, K.Mahendra and B. Syama Sundar* Der Pharma Chemica, 2011, 3(1): 490-496
18. C Cazaubon; J Gougat; F Bousquet; P Guiraudou; R Gayraud; C Lacour; A Roccon; G Galindo; G Barthelemy; B Gautret. J. Pharmacol. Exp. Ther., 1993, 265, 826–834.
19. J.W. Purseglove, E.G. Brown, C.L. Green, and S.R.J. Robbing. Spices, London and New York: Longman 1981; 2, pp. 448-449.
20. Chena W, Lianga Y, Lia H, Xionga Y, Liua X, Wanga G, Xi L Simple, sensitive and rapid LC-ESI-MS method for the quantitation of lafutidine in human plasma-application to pharmacokinetic studies. J Pharm Biomed Anal. 2006 41(1):256–260 PubMed Abstract |Publisher Full Text.
21. Xiang-lin Z, Yun-di Q, Kai-peng L, Qingming D, Xiao L Determination of lafutidine in human serum by HPLC-FD method and study of pharmacokinetics. Chin J Hospital Pharm. CNKI:SUN:ZGYZ. 0.2005-12-01712.

*****